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COMMUNITY STRUCTURE, NUTRIENT DYNAMICS, AND THE DEGRADATION OF DIETHYL PHTHALATE IN AQUATIC LABORATORY MICROCOSMS

by

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16. ABSTRACT

An investigation was conducted of the environmental fate of diethyl phthalate (DEP) in the continuous-flow channel microcosms housed in the USEPA's Environmental Research Laboratory, Athens, Ga. The objectives of the investigations were to determine (1) whether a definable stable state could be achieved, (2) the effects of different nutrient treatments on ecosystem structure and function and on the fate of DEP and (3) the degree of similarity between replicate microcosms.

Aufwuchs assemblages in the microcosms reached fairly stable levels of biomass metabolic activity, and similar species composition within 2 or 3 months after inoculation. Communities receiving direct nutrient inputs appeared to stabilize first, followed by downstream communities. A highly significant relationship between phosphorus inputs and aufwuchs chlorophyll a was established suggesting that the relatively stable input concentrations of inorganic nutrients into any given compartment were among the primary factors controlling maximum development of aufwuchs.

Replicate microcosms were statistically indistinguishable with respect to nutrient concentrations for most of the experimental period. Compartments receiving direct inputs of inorganic nutrients had the most consistent replicability. Although non-taxonomic community structure was generally similar in replicate compartments, some differences were observed in relative species abundance. Sorption, volatilization, and photolysis were insiginficant processes in the fate of DEP.

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SECTION 1

INTRODUCTION

The EXposure Analysis Modeling System (EXAMS) was developed by the Environmental Systems Branch of the USEPA's Environmental Research Laboratory, Athens, Georgia, to provide a tool for predicting the fate of chemical compounds in natural waters (Burns et al. 1981). Since its implementation, the model has been tested with the chemical methyl parathion under controlled laboratory conditions using the Aquatic Ecosystem Simulator (AEcoS), housed at the same laboratory. During 1980, the model was subjected to another experimental test, again in AEcoS, using the chemical diethyl phthalate (DEP). The purpose of the present report is to (1) present the experimental results of the DEP fate study in AEcoS, and (2) evaluate the degree to which the experiments met some of the assumptions implicit in the EXAMS-AEcoS testing procedure. EXAMS modeling results will be presented elsewhere.

ENVIRONMENTAL FATE OF DEP

Phthalic acid esters (PAEs) are plasticizers which are in widespread use today with approximately two million tons produced annually (Thomas et al. 1978). As plasticizers, PAEs are used in PVC plastics, resulting in materials that are flexible and supple. PVC plastics are widely used for wire and cable insulation, construction materials, home furnishings, automotive components, apparel, and medical products. Non-plasticizer uses of phthalates include pesticide carriers, munitions, cosmetics, and insect repellents. Phthalate plasticizers are currently being produced and used in amounts that could easily contribute to environmental pollution. The high disposal rates of plastics may be producing an environmental reservoir of the phthalates that may slowly leach into aquatic ecosystems (Giam et al. 1978). The two most commonly used phthalate compounds are di-n-butyl phthalate (DBP) and di(2-ethylhexyl) phthalate (DEHP). In a recent comparison of concentrations of selected organics in the atmosphere (Atlas and Giam 1981), both DBP and DEHP were found in the atmosphere in amounts above those found for PCB, DDE, lindane, dieldrin, and chlordane.

Limited information exists on the fate of phthalate esters in biological systems or aquatic environments despite their widespread use. PAEs have been identified in soil (Ogner and Schnitzer 1970), fish (Mayer et al. 1972), food and biological samples (Cerbulis and Ard 1967, Zitko 1973), freshwaters of the Mississippi River and marine coastal waters of the Gulf of Mexico (Corcoran 1973), open-ocean atmospheric samples of the Gulf of Mexico (Giam et al. 1980), and waters of the Charles and Merrimack Rivers (Hites 1973). Metcalf et al. (1973) studied the uptake of DEHP by a variety of aquatic organisms using a laboratory model ecosystem with a terrestrial-aquatic interface. This study showed that DEHP is rapidly accumulated by a variety of aquatic plants and animals but is biodegraded very slowly by algae, Daphnia, snails and clams, and more rapidly in fish. The authors suggest that the major degradative pathways are through hydrolysis of the ester groups to produce monoethylhexyl phthalate, phthalic acid, and phthalic anhydride. In support of this, Stalling et al. (1973) have shown that DEHP is metabolized primarily to the mono ester in channel catfish.

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FOREWORD

Environmental protection efforts are increasingly directed towards preventing adverse health and ecological effects associated with specific compounds of natural or human origin. As part of this Laboratory's research on the occurrence, movement, transformation, impact, and control of environmental contaminants, the Environmental Systems Branch studies complexes of environmental processes that control the transport, transformation, degradation, and impact of pollutants or other materials in soil and water and assesses environmental factors that affect water quality.

Concern about environmental exposure to toxic substances has increased the need for accurate information on the transport, fate, and effects of trace contaminants in natural waters. One technique that shows promise as a useful tool for providing some of this information is the use of laboratory microcosms as ecosystems for the assessment of pollutant exposure to natural aquatic systems. This report presents an evaluation of microcosms as test systems for exposure analysis models using a phthalate ester as the study compound.

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ABSTRACT

The research reported herein was conducted in two 8-compartment continuous-flow channel microcosms housed in the USEPA's Environmental Research Laboratory, Athens, Georgia. The microcosms were designed and established to enable testing of the Exposure Analysis Modeling System (EXAMS), a theoretical-type predictive model for the determination of the fate of toxic compounds in freshwater systems.

The objectives of the investigations were to determine (1) whether a definable stable state could be achieved in which to test the model; (2) the degree of similarity between corresponding microcosms; (3) the effects of different nutrient treatments on ecosystem structure and function; and (4) the fate of diethyl phthalate (DEP) in channel microcosms.

Aufwuchs assemblages in the microcosms reached fairly stable levels of biomass, metabolic activity, and similar species composition within two or three months after inoculation. Communities receiving direct nutrient inputs appeared to stabilize first, followed by downstream communities.

Replicate microcosms were statistically indistinguishable with respect to nutrient concentrations for most of the experimental period. Compartments receiving direct inputs of inorganic nutrients had the most consistent replicability. Although non-taxonomic community structure was generally similar in replicate compartments, some differences were observed in relative species abundance.

A highly significant relationship between phosphorus inputs and aufwuchs chlorophyll _a_ was established suggesting that the relatively stable input concentrations of inorganic nutrients into any given compartment were among the primary factors controlling maximum development of aufwuchs.

Sorption, volatilization, and photolysis were insignificant processes in the fate of DEP. Alkaline hydrolysis at pH 10 showed only a slight effect. Microbial degradation was the dominant process. First-order degradation rates were all within an order of magnitude even though there were significant differences in both chemical environments and biological communities.

This report covers the period November 13, 1979, to January 29, 1981, and work was completed as of December 1981.

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Several reports examine the biodegradation of phthalate esters by activated sludge and bacteria. Saeger and Tucker (1976) indicated that 70 to 78% of DEHP was biodegraded in an activated sludge test. Kurane et al. (1980) showed that DBP, DEHP, and dimethy phthalate (DMP) were hydrolyzed to the free phthalic acid in the presence of the bacteria *Nocardia erythropolis*.

DEP is present in the environment in small quantities compared to DBP and DEHP. Background contamination from natural waters, reagents, solvents, and other laboratory materials do not pose significant problems, thus making it well suited for study in an aquatic ecosystem facility such as AEcoS. DEP is commonly used as a denaturant in the preparation of perfumes and insect repellents with limited use as a plasticizer. Little information specific to DEP and its impact on the aquatic environment is available; therefore, the aquatic fate of this compound has been inferred from data for phthalate esters as a group (Versar, Inc. 1979). Based on such data, bioaccumulation and biodegradation are probably the most important biological processes determining the fate of DEP in an aquatic ecosystem. Losses of environmental pollutants can also occur through hydrolysis, volatilization, photolysis, and sorption. However, using the method of Radding et al. (1977), we estimated from the half-life for alkaline hydrolysis that hydrolysis is not an important process. Volatilization of DEP as a transport process can also be considered inconsequential since the vapor pressure of DEP is extremely low (0.05 Torr at 70°C). Only limited amounts of data exist on the hydrolysis, photolysis, and sorption of DEP, and thus it was important to investigate the effects of these processes on the fate of DEP.

The specific objective of the toxicant testing phase of this study was to determine the fate of DEP in a complex aquatic microcosm. Processes of particular interest were sorption, volatilization, photolysis, hydrolysis, and microbial degradation. Thus it was necessary to set up the experimentation such that the rate coefficients of these environmental processes could be determined. Testing was conducted in two compartments to ascertain rates of sorption, volatilization, hydrolysis, and photolysis; microbial degradation was studied under various environmental conditions in the other compartments.

ASSUMPTIONS OF EXAMS and AEcoS

Implicit in the EXAMS parameterization experiments are several assumptions pertaining both to the model and to the experimental facility:

1. The chemical, physical, and biological processes that influence the environmental fate of a compound are at steady state during the time interval under study. Physical conditions (temperature, light intensity, turbulence, etc) and the chemical regime (nutrient, toxicant, and water inputs) in AEcoS remained relatively constant under laboratory control throughout the experiment, except during occasional mechanical failures. However, temporal variability did occur in the biotic communities and in biotically mediated processes. Therefore, the validity of the steady-state assumption was examined with respect to uptake and release of nutrients, diel cycles of metabolic gases, and organic structure of the biotic communities.

2. Manipulation of chemical inputs into various compartments within AEcoS creates a set of different environments in which to study the environmental fate of a compound. To this end, different levels of inorganic and organic nutrient enrichment were applied. Chemical and biological data were analyzed to determine whether the chemical manipulations produced distinct chemical environments and microbial assemblages.

3. Identically treated compartments in AEcoS behave as experimental replicates. Chemical and biological data from duplicate AEcoS channels were statistically analyzed to determine whether similar experimental treatments resulted in replicable behavior.

SECTION 2

CONCLUSIONS

1. Sorption, volatilization, photolysis, and chemical hydrolysis were insignificant processes in the fate of DEP. Microbial degradation of DEP resulted in the disappearance of 36 to 90% of the compound. Although aufwuchs bacterial numbers varied by several orders of magnitude among the microcosms, first-order degradation rates were similar in all except those receiving low inorganic and high organic nutrient inputs. Therefore, only a fraction of the total bacteria present may have participated in DEP degradation.

2. Aufwuchs communities appeared to reach a definable steady state within two to three months of inoculation, based on available data for taxonomic and non-taxonomic community structure (relative abundance of algal species, similarity indices, chlorophyll a, ash-free dry weight, ATP, and total organic carbon) and community metabolic activity (relative changes in dissolved oxygen and pH). Also, based on metabolic activity estimates, communities receiving direct nutrient inputs appeared to stabilize first, followed by downstream communities.

3. A significant linear regression ($r = 0.88$) between phosphorus loading and aufwuchs chlorophyll a suggested that phosphorus inputs were important in controlling non-taxonomic structure. A significant curvilinear relationship ($r = 0.91$) between the same two parameters indicated that spatial limitations may have become important in controlling aufwuchs development at higher phosphorus loading rates.

Non-taxonomic parameters associated with living aufwuchs (chlorophyll a, ATP, and bacterial numbers) provided greater resolution in distinguishing structurally distinct communities than did those that also included detritus (ash-free dry weight and total organic carbon). As expected, microcosms receiving nutrient enrichment displayed significant increases in non-taxonomic structure for all parameters examined.

4. Replicate microcosms were generally similar with respect to both chemical and biological variables. Nutrient concentrations (PO_4, NO_3, NH_3) were statistically indistinguishable for most of the experimental period; the differences which did occur could not be attributed to experimental manipulations. Non-taxonomic structure also was similar between replicate microcosms. High light intensity, temperature, and nutrient loading combined with low carbonate alkalinity appeared to select for euryecious organisms typical of eutrophic systems. Algal species composition was generally more similar than dissimilar (based on SIMI indices) among the different microcosms. The differences which were observed usually resulted from large variations in relative abundances of one or two algal species. Shannon-Wiener species diversity and evenness estimates indicated relatively complex algal aufwuchs communities.

SECTION 3

RECOMMENDATIONS

1. In this study, DEP degradation was observed under environmental conditions typical of eutrophic ecosystems. Further research is needed to characterize the fate of DEP and its metabolites over a broader spectrum of environmental conditions and trophic states.

2. DEP concentrations and subsequent metabolites were assumed to have no antagonistic or synergistic effects on the biologically mediated degradation of the compound. To verify this assumption, future experimental designs should be expanded to include a range of chemical concentrations permitting a simultaneous investigation of compound fate and effects.

SECTION 4

FACILITY

DESCRIPTION AND EXPERIMENTAL DESIGN

Physical Setup

This study was conducted in the EPA's Aquatic Ecosystem Simulator (AEcoS), a controlled-environment chamber that houses a 19.5 m long, 46 cm wide, 51 cm deep "U-shaped" flume. The Plexiglas flume is lined with adhesive-backed 4 mil Teflon film. The flume is divided into two independent channels (designated A and B), each subdivided into eight 250-liter compartments called Continuously Stirred Tank Reactors (CSTR). Each CSTR is equipped with an outlet weir such that the effluent from an upstream CSTR constitutes the influent for the next downstream CSTR. Uniform mixing in each CSTR is accomplished by the use of Teflon-covered Plexiglas paddlewheels suspended longitudinally down the channel and adjusted to desired mixing rates by variable speed controllers. The paddlewheel speed for this experiment was set at 2.0 rpm.

The environmental chamber provides control of air and water temperature, water flow, relative humidity, and light quality and quantity. The water supply is well water treated by reverse osmosis and deionization followed by ultraviolet sterilization, and flow was maintained at 500 liters per day at 20°C during the experiment. The chamber temperature was maintained at 21°C with a relative humidity of 50%. The channel was illuminated with 2000 foot-candles of fluorescent light on a 12 hour light/12 hour dark cycle using a radiant energy system located 0.6 meter above the water surface.

Nutrient chemicals were introduced continuously to selected CSTRs using feed lines of silicone tubing and Harvard peristaltic pumps.

A concentrated solution of the toxicant, DEP, was added using an all glass constant-head system (Figure 1); the metering system was set to 2.0 ml/min to achieve the desired final concentration when diluted with the incoming water.

CSTR Characteristics

Figure 2 shows the experimental conditions of each channel with Channels A and B treated as replicates. The purpose of this design was to estimate the rate of degradation of a toxicant in different environments so each CSTR was designed to simulate different conditions. Concentrations of elements after final dilution in AEcoS are shown in Table 1. The pH adjustments will be discussed in a later section. The purpose of each treatment is summarized in the following sections.

CSTR 1--

Neutral photolysis of DEP. This CSTR received a continuous input of nutrient-free water (345 ml/min) adjusted to a pH of 7.0 with a continuous input of 0.003 M NaOH (4.0 ml/min). CSTR 1 was not inoculated with biotic material and was continuously illuminated with two banks of black-light fluorescent lamps to induce photolysis.

CSTR 2--

Alkaline hydrolysis of DEP. This CSTR received the effluent from CSTR 1 plus a solution of 0.03 M NaOH (4.0 ml/min) to bring the pH to 10.0. CSTR 2 also was not inoculated with biotic material.

CSTR 3--

Effects of "low" nutrient concentrations on community activity. (CSTRs 3-8 were inoculated weekly as described below.) This CSTR received the effluent of CSTR 2 and the input of inorganic nutrient at a flow rate of 4.0 ml/min (Table 2). This CSTR also received an input of HCl to neutralize the base added to CSTR 2. The concentrates were modifications of Freeman's Reference Water (Freeman 1953) with the addition of micronutrients (Miller et al. 1978) and 10% of the total amounts of N and P added to AEcoS (0.062 g NH_4NO_3 and 0.011 g $Na_2HPO_4 \cdot 7\ H_2O$ per liter concentrate).

CSTR 4--

Effect of sediment and low nutrients on DEP degradation. CSTR 4 received only the effluent of CSTR 3. This CSTR contained a stainless steel sediment tray containing 100% unsterilized river sediments (Oconee River, North Georgia).

CSTR 5--

Enhanced microbial degradation of DEP. CSTR 5 received the effluent of CSTR 4 plus 4.0 ml/min of a concentrate of glycerin to stimulate heterotrophic growth. The glycerin was added such that a final concentration of 3.9 mg/l carbon was obtained.

CSTR 6--

Comparison of community development rate with CSTR 3. This CSTR received the effluent of CSTR 5 and an input of high N and P (90% of total amount added to AEcoS). The concentrate was added at the rate of 4.0 ml/min and contained per liter: 0.558 g NH_4NO_3 and 0.101 g $Na_2HPO_4 \cdot 7\ H_2O$.

CSTR 7--

Effects of high amounts of N and P on the rates of community development. This CSTR received only the effluent of CSTR 6.

CSTR 8--

The influence of a natural sediment and high nutrient regime on the fate of DEP. This CSTR received the effluent of CSTR 7 and contained a sediment tray of 100% river sediments (same as CSTR 4).

Inoculation

Biological communities were established in CSTRs 3-8 by inoculating each CSTR with a 1-liter sample from each of two local ponds and a creek. Each replicate CSTR was then cross inoculated with a 1-liter sample from the corresponding CSTR. Reinoculation and cross inoculation were performed weekly throughout the experiment.

pH Adjustment

Experimentation was initiated on November 13, 1979. Nutrient inputs were set up as described in the previous section and weekly inoculations began. There was no addition of DEP from November 13, 1979, to May 23, 1980, to allow time for communities to become established. The addition of Na salts to CSTR 3 during this period of time required the addition of 0.252 meq/l HCl for neutralization. DEP addition began on May 23, 1980. At this time it was necessary to set up pH adjustments for CSTRs 1, 2, and 3. CSTR 1 required the addition of NaOH at a final concentration of 0.034 meq/l to adjust the inflow water pH from 5.8 to 7.0. CSTR 2 required an input of NaOH such that a final concentration of 0.358 meq/l NaOH was maintained to adjust the pH to 10. This required an additional input of 0.358 meq/l HCl to CSTR 3 to neutralize the base added to CSTR 2. In an attempt to lower the overall pH in CSTRs 3-8, an excess of 0.358 meq/l HCl (final concentration) was added to CSTR 3. This excess acid was omitted after a period of 11 weeks in Channel B and 13 weeks in Channel A.

DEP Experimental Design

Initial investigation of the degradation of DEP showed 30-80% losses in any individual CSTR containing a biological community. This made it impossible to study the degradation of DEP in more than one pair of replicate CSTRs (e.g., A3 and B3) at a time. For this part of the study toxicant input was sequentially moved downstream from one CSTR to the next on a weekly schedule.

An aqueous solution containing 34.3 mg/l DEP was dripped in at a rate of 2.0 ml/min; analysis of the final dilution gave a mean input concentration of 194 ug/l DEP.

After this part of the DEP study was completed, a separate study was initiated to determine the sorption, volatilization, photolysis, and hydrolysis of DEP in CSTRs 1 and 2 (in the absence of biological communities). For sorption and volatilization all CSTRs were filled with nutrient-free water. A continuous DEP input of 34.3 mg/l was added at a rate of 2.0 ml/min to CSTR 1 to achieve a final DEP concentration of 200 ug/l; analysis of this final dilution gave a mean input concentration of 191 ug/l DEP. The effluent from each CSTR was the influent for the next downstream CSTR. Samples from CSTRs 1-4 were analyzed in duplicate for five days to determine the amount of volatilization and/or sorption of DEP to the surfaces of each CSTR.

In order to determine the rates of photolysis and hydrolysis, pH adjustments were set up as previously described for CSTRs 1 and 2 and the black light fluorescent lamps were used. The input level of DEP into CSTR 1 remained 191 ug/l. Samples were analysed in duplicate for five days to determine the rates of photolysis in CSTR 1 and rates of hydrolysis in CSTR 2.

SECTION 5

MATERIALS AND METHODS

SAMPLING

Chemical

From November 20, 1979 to July 18, 1980, chemical measurements were routinely performed in each CSTR four times per week: Tuesday and Friday at 0800 hrs (after 11 hours of dark) and Monday and Thursday at 1500 hrs (after 6 hours of light). Beginning with the July 22 additions of DEP, chemical samples were taken in duplicate each Tuesday at 0800 hrs and at 1500 hrs. Table 4 gives a summary of major sampling dates for the entire DEP experiment. From August 5 to October 14 all chemical parameters were measured in triplicate for the designated CSTRs. Oxygen and pH were measured in situ. Water samples for toxicant analyses were pipetted directly from designated CSTRs and extracted immediately in culture tubes with iso-octane. For other chemical parameters (nitrite, nitrate, ammonia, dissolved Kjeldahl nitrogen, total dissolved phosphorus, orthophosphorus, and organic carbon), water was collected with a glass tube, filtered through Gelman HT-450 filters (0.45 um pore size), and dispensed for analyses.

Biological

Aufwuchs samples were collected in triplicate beginning August 5, 1980, using Teflon-covered plastic strips that had been colonized in AEcoS for four weeks prior to sampling. Measured areas of each strip were scraped, brought to exact volume with distilled water, and blended for 5 seconds; aliquots of the resultant slurry were dispensed for algal identification/quantification, total microbial ATP concentration, chlorophyll *a* content, ash-free dry weight, toxicant concentration, bacterial enumeration via plate counts, and total organic carbon.

Suspended samples were collected in triplicate using a 38.0-mm (I.D.) glass column. Samples were blended for 5 seconds and aliquots were dispensed for chlorophyll *a* content, total microbial ATP concentration, and bacterial enumeration via plate counts.

The sediment communities in CSTRs 4 and 8 were sampled in triplicate by collecting shallow (0.5 cm) and deep (2.5 cm) wire baskets contained in the sediment trays. The surface algal mat was removed from the collected sediment samples, the material from the sediment baskets was mixed with a stainless-steel spatula, and aliquots dispensed for analysis of chlorophyll *a* concentration, total microbial ATP concentration, bacterial enumeration via plate counts, and toxicant concentration.

CHEMICAL ANALYSES

DEP

Concentrations of DEP in the water phase were determined according to methods used in earlier studies with malathion (Paris et al. 1975) and methyl parathion (Holm et al. 1981). Analyses were done on a Tracor 550 gas-liquid chromatograph equipped with a Ni-63 electron capture detector and a 2 m (4 mm i.d.) glass column packed with 3% SE-30 on 80/100 mesh Gas-Chrom Q. Concentrations of DEP in aufwuchs and sediment samples were determined on a Tracor MT-222Q gas-liquid chromatograph with a Hall detector using a method described by Holm et al. (1981) for methyl parathion. Samples of aufwuchs were scraped from Teflon strips and extracted directly in methylenechloride with a Brinkman Polytron blender. DEP was extracted from the sediments using a soxlet extraction method (Payne and Benner 1981). The samples were initially extracted with acetonitrile and diluted to volume in hexane for analysis.

Phosphorus and Nitrogen

Ammonia nitrogen (NH_3-N), nitrite nitrogen (NO_2-N), nitrate nitrogen (NO_3-N), orthophosphorus (PO_4-P), total dissolved phosphorus (TDP), and dissolved Kjeldahl nitrogen (DKN) were analyzed in sample filtrates using EPA methods (1979) and a Technicon AutoAnalyzer II system.

Carbon

Total organic carbon (TOC) and dissolved organic carbon (DOC) were determined directly using a Beckman 915-B TOC Analyzer. The method was based upon the organic carbon combustion-infrared method outlined in APHA (1976). Samples for DOC were filtered through Gelman HT-450 filters. All samples were acidified with 50% aqueous HCl and sparged with N_2 gas.

pH and Oxygen

Oxygen and pH were measured in situ with an Orbisphere Model 2708 dissolved oxygen meter and a computer interfaced Beckman Model 941 pH monitoring system, respectively.

BIOLOGICAL ANALYSES

AFDW

Ash-free dry weight was measured according to the procedure outlined for periphyton (APHA 1976). Ash-free dry weight of the aufwuchs slurry samples, expressed in mg/ml slurry, was converted to mg/cm^2 of surface area sampled.

ATP

Total microbial biomass of aufwuchs slurry samples and sediment samples was determined by analysis of adenosine triphosphate (ATP) content of cell extracts using the extraction method of Bancroft et al. (1976) and assayed for ATP with aged firefly lantern extract (FLE-50, Sigma Chemical Co.) using a Science Applications Inc. (Model 3000) photometer (Holm et al. 1981).

A known volume of suspended sample was filtered on a 47 mm Millipore membrane filter (pore size 0.45 um), placed in a beaker, and extracted with boiling tris buffer (pH 7.75, 0.05 M) (APHA 1976). Sample extracts were then analyzed as described above.

Results were expressed as ng/l for the suspended community samples, ng/g dry weight for the sediment community samples, and ng/cm^2 for the aufwuchs samples.

Chlorophyll a

Algal biomass of aufwuchs, suspended, and sediment communities was determined by fluorometric measurement of chlorophyll a (chl a) content corrected for phaeophytin a (Strickland and Parsons 1968). Samples were extracted with acetone using a B. Braun cell homogenizer and analyzed using a G.K. Turner and Associates (Model III) fluorometer (Holm et al. 1981).

Results were expressed as ug/ml for the suspended samples, ug/g dry weight for the sediment samples, and ug/cm^2 for the aufwuchs community.

Algal Species Counts

Algal enumeration and identification was based upon the inverted microscope method of Utermohl as described by Lund et al. (1958). A Zeiss invertoscope D with brightfield and phase contrast capabilities was used for observation and identification of algal species. Samples of the aufwuchs slurry were fixed with a modified acid Lugol's solution (Holm et al. 1981), diluted to a known volume, and settled onto a sedimentation slide for enumeration. Algal density was estimated from observation of 400 algal units or more of the total sample and at least 100 cells of the most abundant species. Results were expressed as algal $units/cm^2$.

Bacterial Plate Counts

Total bacteria were measured using standard plate counts (APHA 1976).

Total Organic Carbon

Before analysis, slurry samples from the aufwuchs community were blended for one minute to resuspend the community. A sample of this suspension was taken and diluted to a workable concentration. The sample was then acidified to $pH < 2$ with 50% aqueous HCl and sparged for 3-5 minutes with N_2 gas. At the time of analysis samples were sonicated for 30 seconds to assure a homogeneous mixture. Samples were analyzed as described in the chemistry section.

NUMERICAL AND STATISTICAL ANALYSES

Algal Species Diversity (Evenness and Similarity)

Aufwuchs algal species diversity (H') was determined from the Shannon-Weiner equation (Taylor et al. 1979).

$$H' = -\sum_{i=1}^{S} P_i \log_2 P_i \qquad (1)$$

where P is the proportion of the i-th taxon in the sample, estimated from n_i/N; n_i is the number of algal units/cm^2 of the i-th taxon; N is the total number of algal units/cm^2 in the sample; and S is the total number of taxa in the sample.

Aufwuchs algal species evenness (J) was calculated from the ratio H'/H_{max} (Taylor et al. 1979) where H_{max} is the diversity obtained if all taxa in the sample are equally abundant and is estimated from $\log_2 S$ (S is the total number of taxa in the sample).

SIMI Indices

The community structure of selected aufwuchs algal samples was compared by means of a similarity index (SIMI). SIMI has a maximum value of 1.00 when algal assemblages are identical between two samples and a value of 0.00 when assemblages are totally different in composition. The formula is as follows (Sullivan 1975).

$$\mathrm{SIMI} = \frac{\sum_{i=1}^{S} P_{ij} \; P_{in}}{\sum_{i=1}^{S} P_{ij}^{\,2} \; \sum_{i=1}^{S} P_{in}^{\,2}} \qquad (2)$$

where P_{ij} and P_{in} are the average relative abundances of the i-th species in the j-th and n-th communities and S is equal to the number of species.

Aufwuchs Biomass Analysis

Data from five estimates of autotrophic and/or heterotrophic components of the aufwuchs community collected during DEP testing (chlorophyll a, microbial ATP, ash-free dry weight(AFDW), total organic carbon (TOC), and total bacterial numbers) were used in the following statistical analyses.

1. Two sample Student t-tests evaluated CSTR replicability of Channel A versus Channel B.

2. One way analysis of variance and Student-Newman-Keuls multiple range test evaluated CSTR treatment differences using combined data from Channels A and B.

3. A matrix of product-moment correlation coefficients using combined data from Channels A and B.

4. The Mann-Whitney test, a nonparametric analogue of the two sample Student t-test, was used to evaluate treatment differences between CSTR 3 and CSTR 6 based on data collected during the entire year.

5. Confidence intervals ($\alpha = 0.05$) were constructed for graphic presentation.

Because standard deviations were proportional to the means, data were transformed by $X' = \log_{10} (x+1)$ before parametric analyses were performed. See Zar (1974) for a complete explanation of test statistics used.

Chemical Environment Analysis

Concentrations of DEP in the water and first-order degradation rate constants were analyzed using the Statistical Analysis System (SAS) (Service 1972), and treatment differences were summarized using Duncan's multiple range test. Differences between mean input level of DEP and concentrations found during the sorption, photolysis, and hydrolysis experiments were evaluated with two sample student t-tests. Student t-tests were used to test chemical replicability of Channels A and B by comparing mean concentrations of NO_3-N, NH_3-N, and PO_4-P for each channel from selected time periods during the sampling year. Differences between morning and midday nutrient mean concentrations were also evaluated by Student t-tests after combining data from Channels A and B.

SECTION 6

RESULTS

DIETHYL PHTHALATE

Concentrations of DEP found in CSTRs 1-4 in the absence of microbial growth and nutrient additions (including pH adjustments) are shown in Table 5. Results indicate that there was no significant loss of DEP resulting either from sorption to channel surfaces or from volatilization. No detectable concentration of DEP was found in the aufwuchs community based on a total of 60 samples extracted and analyzed by the method described. A total of 36 sediment samples extracted and analyzed also showed no detectable concentrations of DEP indicating that after the addition of DEP to CSTRs containing microbial communities, no sorption to the sediments or microorganisms occurred. There was no measurable loss of DEP by photolysis in CSTR 1 while the loss of DEP from water as a result of chemical hydrolysis at pH 10 in CSTR 2 was 4-5% (Table 5). The percent hydrolyzed was calculated using the output from CSTR 1 as the input to CSTR 2.

Concentrations of DEP in CSTRs containing microbial growth indicated that rapid loss of DEP occurred due to microbial degradation (Table 6). The first-order rates of loss, k_1, for all CSTRs were calculated as follows (Lewis et al. 1981).

$$k_1 = \frac{L/V - (F/V)\,C}{C} \tag{3}$$

where L is the mass of DEP added to the CSTR per time (mg/hr), V is the volume of the CSTR (liters), F is the flow rate of the CSTR (l/hr), and C is the steady-state concentration (mg/l) of DEP determined experimentally in the CSTR. Table 6 shows a summary of the rate constants calculated in this manner. Samples were taken for analysis beginning on September 9, 1980.

PHOSPHORUS CONCENTRATIONS

Orthophosphate

Figures 3-5 summarize the orthophosphate concentrations in CSTR 3, 4, and 5 over the entire course of the DEP experiment.

CSTRs A3 and B3 both exhibited rapid initial uptake of phosphorus; seven days after inoculation, PO_4-P concentrations had dropped from a mean of 0.017 mg/l to 0.008 mg/l in CSTR A3 and 0.002 mg/l in CSTR B3. Concentrations then became relatively stable, fluctuating between 0.002 mg/l and 0.006 mg/l in both CSTRs. During March 1980 PO_4-P concentrations in both CSTRs increased and exhibited much wider fluctuations, particularly in CSTR B3.

Between March and June, PO_4-P concentrations in CSTRs A3 and B3 (Figure 3) showed wide fluctuations, but became relatively stable by July. Concentrations in both CSTRs remained at or below 0.006 mg/l until the end of the experiment. Neither CSTR reacted noticably to the cessation of acid additions in August or the readditions in October and no effects were noticed from the additions of DEP.

The PO_4-P concentration graphs for CSTRs 4 and 5 (Figures 4 and 5) are very similar to each other and to CSTR 3 (Figure 3). Notable increases in PO_4-P concentrations around late March and early June 1980 in CSTR 4 and CSTR 5 were most likely due to the PO_4-P increases in CSTR 3.

The PO_4-P concentration graphs for CSTRs 6, 7, and 8 (Figures 6-8) reveal higher standing concentrations, as well as a wider range of fluctuations than in CSTRs 3, 4, and 5. In CSTR 6, as in CSTR 3, initial utilization of PO_4-P was rapid, with concentrations falling from a mean of around 0.130 mg/l to below 0.030 mg/l within 2 weeks of inoculation. Concentrations fluctuated during the next two months but remained below 0.060 mg/l. After January 20, 1980, concentrations increased slightly but remained below input levels. The increasing trend continued during April and May. Observed peaks in PO_4-P concentrations in CSTR 6 at the end of May may have been due to acid additions. This transient response was particularly notable in CSTR A6. PO_4-P concentrations increased until early July when concentrations in CSTR A6 dropped noticeably below those in B6. Concentrations increased in both CSTRs during the last month (November 1980) of the experiment. Again, there was no apparant response to later acid manipulations or DEP treatments. PO_4-P concentrations in CSTRs 7 and 8 generally showed temporal patterns similar to those in CSTR 6.

Total Dissolved Phosphorus

Total dissolved phosphorus (TDP) concentrations (Figures 9-14) include orthophosphate, hydrolyzable, and dissolved organic phosphorus. The TDP graphs for CSTRs 3-5 (Figures 9-11) demonstrate the same trends as the PO_4-P graphs (Figures 3-5) discussed in the previous section. TDP concentration values were similar to the PO_4-P concentration values reflecting low dissolved organic phosphorus in these CSTRs.

TDP concentration graphs for CSTRs 6-8 (Figures 12-14) followed the same trends as the PO_4-P concentration graphs (Figures 6-8). These high nutrient CSTRs, however, contained a greater percentage (> 20%) of dissolved organic and/or hydrolyzable phosphorus.

NITROGEN CONCENTRATIONS

Nitrate Nitrogen

Figures 15-20 present NO_3-N concentrations over time in all CSTRs. NO_3-N concentrations in CSTR 3 dropped rapidly from the input value of 0.12 mg/l to below detection limits at the start of the experiment (Figure 15), remained at this level for about two weeks, and then increased to between 0.02-0.06 mg/l where they remained for more than half of the experimental period. During the last 5 months of the experiment NO_3-N concentrations increased to an average of 0.09 mg/l by December 1980. In CSTRs 4 and 5, NO_3-N concentrations fell below detection limits after inoculation, but gradually increased during the experiment (Figures 16 and 17). By December 1980, NO_3-N in CSTR 4 averaged 0.04 mg/l; except for transient peaks, concentrations in CSTR 5 never exceeded 0.02 mg/l. In CSTR 6, NO_3-N concentrations decreased from the input value of 1.12 mg/l over a period of 3 months, reaching a minimum of 0.50 mg/l in February (Figure 18). Concentrations gradually increased over the next 4 months to approximately 1 mg/l and remained near that level for the rest of the experiment. Channel A showed a variation from this pattern only during May 1980 when acid additions began. NO_3-N concentrations in CSTRs 7 and 8 (Figures 19 and 20) followed the same pattern as in CSTR 6, decreasing to a minimum of 0.40 mg/l in February and then approaching input levels.

Nitrite Nitrogen

Nitrite nitrogen was not introduced into AEcoS as a nutrient and consequently any accumulation of NO_2-N was due to chemical or biological activity. This would usually be in the form of ammonia oxidation, nitrate reduction, or excretion by organisms (Mortonson and Brooks 1980). No accumulation of nitrite was observed during the DEP experiment in the low nutrient CSTRs 3-5.

In CSTRs 6-8 nitrite accumulated to concentrations exceeding those usually found in natural systems. Figure 21 shows that nitrite concentrations in CSTRs 6 began to accumulate within 2 weeks and steadily increased to a maximum of 0.40 mg/l NO_2-N in CSTR A6 and 0.46 mg/l in CSTR B6 in March. Concentrations then declined until both CSTRs reached 0.02 mg/l in May. In June CSTR A6 showed a sudden increase to 0.39 mg/l that then declined to low levels in early June; no increase was observed in CSTR B6 during this period. For the remaining 5 months of the experiment, NO_2-N levels in CSTRs A6 and B6 remained relatively low (0.05 mg/l).

Nitrite accumulation also was observed in CSTRs 7 and 8 (Figures 22 and 23). Compared to CSTR 6, slightly higher levels of NO_2-N were reached in CSTRs 7 and 8 during the mid-March maximum. CSTRs A7 and A8 behaved similarly to CSTR A6 in June, and both CSTRs maintained relatively low NO_2-N levels for the remainder of the experiment.

Ammonia Nitrogen

In CSTR 3, NH_3-N concentrations dropped rapidly from the input value of 0.125 mg/l to between 0.010-0.040 mg/l and remained at this level for almost the entire experimental period (Figure 24). Channel A showed a variation from this pattern during late May, when there was a transient increase in the standing concentration to 0.45 mg/l; levels returned to previous low values (0.040 mg/l) within two weeks. Channel B showed increased NH_3-N concentrations during May and August, but returned to previous levels by the end of each month. NH_3-N values in CSTRs 4 and 5 dropped to below 0.040 mg/l and generally remained at this level throughout the experiment (Figures 25 and 26), except for a temporary increase during May in CSTR A4.

Input of NH_3-N into CSTR 6 was 1.125 mg/l. Average concentrations in the water were 0.5 mg/l (morning) and 0.2 mg/l (midday) for the first four months of the experiment (Figure 27). In March, these values decreased to below 0.300 mg/l (morning) and below 0.010 mg/l (midday), but after two months, returned to previous levels and remained relatively stable for the rest of the experiment. Channel A showed an increase in NH_3-N concentrations to a maximum of 1.10 mg/l during the end of May, but returned to previous levels by the next week. In CSTRs 7 and 8, NH_3-N levels were lower than in CSTR 6, but generally showed the same temporal patterns (Figures 28 and 29).

Dissolved Kjeldahl Nitrogen

Dissolved Kjeldahl nitrogen (DKN) in CSTR 3 remained near 0.2 mg/l throughout the experiment (Figure 30). Channel A showed an increase during May, due to the previously mentioned increase in NH_3-N. In CSTRs 4 and 5 (Figures 31 and 32), there was no obvious trend in the data; values remained near 0.2 mg N/l.

Dissolved Kjeldahl nitrogen remained near 0.6-0.8 mg/l in CSTR 6 (Figure 33); Channel A also reflected the increase in NH_3-N during May.

In CSTRs 7 and 8 (Figures 34 and 35), concentrations in the water showed high week-to-week variability, but generally remained between 0.3 and 0.9 mg/l. Channel A showed the same pattern of increase in May that was observed in CSTR 6.

CARBON CONCENTRATIONS

Dissolved Organic Carbon

Dissolved organic carbon (DOC) concentrations remained fairly constant throughout the experiment at levels dependent on nutrient treatments (Figures 36-41). In CSTRs 3 and 4, DOC concentrations averaged about 1 mg/l with peaks as high as 4 mg/l. In the last month of observation, concentrations increased rapidly to about 6 mg/l in the midday samples. CSTR 5 received a continuous input of 3.9 mg/l DOC (as glycerin) and concentrations remained near this level throughout the experiment, with peaks up to 9.0 mg/l in May and August. Midday DOC values again increased during the last month.

In CSTRs 6-8, DOC concentrations averaged about 2 mg/l and showed a steady increase to about 3 mg/l during the last four months. From July on less carbon analyses were performed due to instrument break-down. As in previous CSTRs, midday DOC concentrations rapidly increased to about 6 mg/l during the final month of the experiment.

Total Organic Carbon

Total organic carbon (TOC) concentrations (Figure 42-47) were considerably more variable in all CSTRs than were DOC concentrations, probably due to sampling errors and to non-homogeneous distribution of suspended particles. In CSTRs A3 and A4, TOC was about 2 mg/l for much of the experiment, but with major peaks as high as 12 mg/l. CSTRs B3 and B4 showed wider variations, especially after March. TOC concentrations in CSTR 5 were higher (5 mg/l) due to the glycerin input, but the temporal patterns were somewhat similar to those in CSTRs 3 and 4.

From the beginning of the experiment until around April, TOC concentrations averaged about 2 mg/l in CSTR 6, followed by wide variations especially notable in A6. TOC levels were slightly higher (average approximately 3 mg/l) in CSTRs 7 and 8. TOC variability in all CSTRs accompanied development of the aufwuchs communities and apparently resulted from sloughing of particulate organic matter.

DISSOLVED OXYGEN

As is typical in aquatic ecosystems, dissolved oxygen (DO) concentrations (Figures 48-53) showed daily cycles associated with photosynthetic and respiratory activity in the CSTR communities. Following an initial peak to about 15 mg/l in early December associated with a bloom of suspended algae (unpublished data), midday DO values remained relatively stable (10-13 mg/l) until May in all CSTRs except 4 and 5. In the latter systems, midday DO gradually increased from about 9 mg/l to 13 mg/l until May, and then fluctuated between 8 and 15 mg/l. Morning DO concentrations remained fairly stable throughout the experiment in CSTRs 3, 4, and 6, gradually increased in CSTR 5, and gradually decreased in CSTRs 7 and 8.

Spikes in both midday and morning DO concentrations in May and June were due to extended periods of darkness and light, respectively, caused by malfunctions in the light system. The low midday values at the end of May coincided with the initiation of acid/base additions.

Hydrogen Ion Activity (pH)

In the absence of acid/base additions to the channels, temporal pH patterns (Figures 54-59) were relatively stable and closely resembled those described for dissolved oxygen. This is due to the opposite metabolic behaviors of O_2 and CO_2, pH being an inverse function of the latter. pH generally ranged from as low as about 6.5 in the morning to as high as about 10.2 in the evening; this wide range is probably due to intense metabolic activity coupled to the low alkalinity (25 mg/l $CaCO_3$) of the medium.

During the first period of acid/base addition (May - August), temporal pH patterns were obscured and replaced by approximately 7-day cycles of increasing and decreasing pH. After acid/base additions were stopped in August, diel pH patterns once again became similar to those of dissolved oxygen. Similar results occurred during and following the second acid/base additions in October and November.

Several spikes in the pH data were due to mechanical malfunction of the lighting system during mid-January, early May, and mid-September.

A more detailed evaluation of the acid perturbations is discussed by Kollig and Hall (1982).

Aufwuchs Communities

At the time of DEP experimentation (July - November), temporal development of the biotic community had progressed to the point where profuse aufwuchs growth covered all colonizable surfaces in the channel microcosms. The development of an aufwuchs dominated community in "mature" microcosms has been noted in previous studies (Dudzik et al. 1979) and is primarily a consequence of the high surface area to volume ratios typically found in microcosms.

The approximate values and percentages of chlorophyll a, ATP, and bacterial numbers found in the sediment, suspended, and aufwuchs assemblages of CSTRs 4 and 8 are presented in Table 7. Due to the large contribution of the aufwuchs assemblage to total system biomass and to the observation that the species composition of the suspended and aufwuchs algal assemblages was generally very similar in any given CSTR (unpublished data), the present evaluation is limited to analysis of aufwuchs data.

The aufwuchs assemblage biomass and species composition data are summarized in Figures 60-64 and Tables 8-16. In addition, trace metal analysis of the aufwuchs from each of the CSTRs was done during the week of December 2, 1980. Results of these analyses are presented in Table 17. Evaluations of the results are found in the Discussion section.

SECTION 7

DISCUSSION

ENVIRONMENTAL FATE OF DEP

Results of this study indicate that sorption, volatilization, photolysis, and chemical hydrolysis were insignificant processes in the fate of DEP. Student's t-tests conducted on data shown in Table 5 showed no differences between the mean input level of DEP and the concentrations found in the CSTRs for sorption/volatilization ($\alpha = 0.05$). A similar evaluation of the photolysis data showed no significant differences. Evaluation of the data on alkaline hydrolysis of DEP at pH 10 showed only a slight effect. However, since it has been shown that the half-life of DEP resulting from alkaline hydrolysis is proportional to hydroxyl concentration (Wolfe et al. 1980), and since the pH in AEcoS ranged from 6.5 to 9.5, it can be concluded that alkaline hydrolysis was an insignificant process. These results corroborate the conclusions of Versar, Inc. (1979).

Results indicate that microbial degradation of DEP was the dominant process in AEcoS. Table 6 shows that DEP was degraded from 36-90% in CSTRs containing biological communities. It has been previously determined (Lewis et al. 1981) that bacterial transformation is by far the most significant process in determining the fate of DEP in aquatic ecosystems. Figure 65 shows the first-order rates of degradation, k_1, for DEP in AEcoS. These data indicate that the rates in all CSTRs were very similar with the exception of CSTR 5 (Duncans Multiple Range Test, $\alpha = 0.05$) even though there were significant differences in both chemical environments and aufwuchs biomass levels (Tables 8 and 18). Figure 65 further demonstrates that all rates were within an order of magnitude which was surprising considering the large differences in the biological communities.

Although the concentrations of bacteria in the CSTRs varied by several orders of magnitude (Figure 64), there is some indication that only a fraction of the total bacteria present participated in the degradation of DEP. This conclusion has been supported in an earlier study by Lewis and Holm (1981) for both methyl parathion and diethyl phthalate in AEcoS. It also has been shown by Lewis et al. (1981) that the rates of transformation in aufwuchs dominated systems can be predicted within an order of magnitude using second order rate coefficients based on total bacterial plate counts of batch cultures. Any differences between predicted and observed rate values may be attributable to the fact that only a percentage of the total bacteria participated in the biotransformation of DEP. The need exists for further investigation of the role of bacteria in biodegradation in terms of percent degraders and of the precision and accuracy with which first-order rates can be predicted from second-order rates using bacterial plate counts.

ASSUMPTIONS OF EXAMS AND AEcoS

Evaluations of Steady-State

The concept of ecological succession toward some climax or steady state biotic community has long been a subject of discussion in the ecological literature. Despite numerous debates about the mechanisms, directionality or deterministic nature of the process, it is frequently suggested that under constant environmental conditions, ecological succession results in biotic communities of relatively constant species composition over some period of time. Furthermore, this constancy of community structure is presumably reflected in measures of overall community function, such as nutrient retention and gross productivity.

Several qualitative models have been proposed that detail expected patterns of change as ecosystems develop toward steady state (Margalef 1968, Odum 1971, Vitousek 1977). Empirical tests of these predictions have been attempted in natural ecosystems, but long successional time scales and variable environmental conditions have precluded firm conclusions. Investigations in laboratory microcosms that approach steady state under controlled environmental conditions, however, have lent support to a number of the predictions (Gordon et al. 1969, Cooke 1977, Wilhm and Long 1969, Kurihara 1978).

Since the EXAMS model rests on the assumption of steady state, and since the AEcoS system is used to validate EXAMS, it is important to determine whether AEcoS achieves an operationally definable steady state within experimental time frames.

An attempt was made to make such a determination with data from the DEP experiment. This consisted of visual and, where possible, statistical analysis of the chemical and biological data. Temporal patterns of nutrient retention, dissolved metabolic gas concentrations, and organic structure were considered over the entire experimental period.

Nutrient Retention

Ecosystems can be considered to be at steady state when total nutrient output is equal to total nutrient input (Jordan and Kline 1972; Vitousek 1977). In the present study, total input concentrations were relatively constant for all elements. System outputs were measured as standing dissolved concentrations times output volume of each CSTR. Since particulate outputs were not measured, complete nutrient budgets could not be calculated from the data; interpretations are therefore tentative. However, output/input ratios were calculated for dissolved PO_4-P, NO_3-N, and NH_3-N to provide an estimate of retention patterns for these chemical species.

Phosphate was rapidly and almost completely utilized by communities under both low (CSTR 3, Figure 66) and high (CSTR 6, Figure 67) nutrient regimes. The latter communities thus incorporated approximately ten times as much PO_4-P as the former, as reflected in significantly higher biomass levels in CSTR 6 (Table 8). The output/input ratio remained low for most of the experiment in CSTR 3 but gradually increased toward unity near the end of the experiment in CSTR 6. The increase in suspended particulate organic matter mid-way through the experiment (Figures 42-47) suggests that PO_4-P (as well as other dissolved nutrients) comprised only a portion of the total output and, therefore, the total output/input ratio may have approached unity. Net system loss of PO_4-P (i.e., output > input) also is plausable since the communities in some CSTRs showed signs of senescense (increased concentrations of phaeo-pigments and $P/R < 1.0$, unpublished data) late in the experiment.

Initial uptake of NO_3-N (Figure 68) and NH_3-N (Figure 70) in CSTR 3 was rapid, probably corresponding to a bloom of suspended algae. Outputs of NO_3-N then gradually increased through time, approaching input concentration by the end of the experiment in CSTR A3. NH_3-N outputs remained low for most of the time, however, possibly indicating net uptake of nitrogen, preferentially in the form of NH_3-N. CSTR 6 (Figures 69 and 71) demonstrated slight retention of NO_3-N during the first half of the experiment but continuous uptake of NH_3-N for the entire period of observation (except in response to acid/base additions); preferential use of NH_3-N is again indicated.

In the absence of particulate import and export estimates, it is not possible to determine whether the communities achieved steady state in terms of nutrient retention. In some cases (PO_4-P in CSTR 3 and NH_3-N in CSTRs 3 and 6), however, there were periods of time in which the rate of nutrient uptake remained relatively constant, especially during the early months of the experiment. If, as indicated in Figures 60-64, total community biomass generally reached a maximum level after about 2 months of development, then particulate loss may have become an important factor relatively early, possibly resulting in nearly equal total output and input nutrient concentrations.

Metabolic Activity

Measurements of dissolved oxygen (DO) were made weekly at lights-on and 6 hours later at midday. Diel oxygen curves measured on several occasions (unpublished data) showed that after 6 hours of light, DO concentrations reached about 75% of the daily maximum. Therefore, the difference between midday and morning DO concentrations provides a relative comparison of net daytime productivity, uncorrected for diffusion and accrual.

In general, all CSTRs (Figures 48-53) except 5 showed an initial burst in oxygen production associated with the early bloom of suspended algae. After about a month, production rates declined somewhat and approached fairly stable levels in CSTRs receiving nutrients either directly (CSTRs 3 and 6) or in large amounts from upstream (CSTRs 7 and 8). Low nutrient CSTRs 4 and 5 required longer periods of time to reach stable rates of oxygen production and never achieved rates as high as the others. Additions of glycerin had little apparent effect on oxygen metabolism in CSTR 5; heterotrophic activity was probably limited by the low concentrations of inorganic nutrients reaching this community.

In the absence of acid/base additions, diel pH values (Figures 54-59) showed trends at least qualitatively similar to those described for dissolved oxygen.

CSTRs 3 and 6 reached stable daytime production rates after about two months of operation. Without measurements of corresponding rates of nighttime respiration, however, ecological steady state (P/R = 1.0) cannot be conclusively defined. Diel oxygen curves measured during July 1980 revealed P/R $\geq$ 1.0 in all CSTRs, and during November 1980 P/R < 1.0 in CSTR 6 (unpublished data). In static microcosms P/R typically approaches unity within 30-60 days (Wilhm and Long 1969; Odum 1971), but flow through systems may require longer periods of time (Hendrix et al. 1981).

Aufwuchs Biomass

A highly significant relationship between total dissolved phosphorus input concentrations and aufwuchs chlorophyll *a* concentration was established from data collected during DEP additions. This empirical relationship suggests that the relatively stable input concentrations of inorganic nutrients into any given CSTR were primary factors controlling maximum development of aufwuchs biomass; Wilhm and Long (1969) demonstrated a similar phenomenon in static microcosms. The overall stability of aufwuchs biomass in all CSTRs during the sampling year, as measured by chlorophyll *a*, ATP, AFDW, TOC, and bacterial numbers, is shown in Figures 60-64. "Early" data points represent the mean values of samples collected on 1/8/80 and 1/22/80 (n = 4), "mid" data points from 5/27/80 (n = 2), and "late" data points during DEP additions (8/5/80 - 9/16/80, n = 6). These three time periods roughly correspond to 2, 6, and 9 months of system development.

With the exception of the "early" TOC data, the 95% confidence intervals around the mean biomass estimates for any given CSTR during DEP additions mostly include the mean values from the two earlier time periods; this observation suggests that aufwuchs assemblages attained a relatively stable biomass level approximately 2 months after initial inoculation and maintained this stability throughout the experiment. The rather wide confidence intervals around these estimates of community structure are functions of the inherent spatial variability in aufwuchs distribution and associated sampling problems, most notably loss of material from sloughing. This high variability becomes particularly problematic when rigorous statistical evaluation of changes in structural parameters is attempted. In a recent study using artificial streams, Rodgers et al. (1979) concluded that measured functional parameters (metabolic estimates) were consistantly less variable and therefore more sensitive to statistical evaluation than measured structural parameters. Although the functional parameters measured in the present study (changes in pH and O_2) could not be adequately resolved into quantitative metabolic estimates, it is apparent from the available data that inclusion of metabolic estimates in future studies could greatly enhance the ability to define system level stability.

Aufwuchs Algal Species Composition

In an attempt to evaluate the temporal stability of algal aufwuchs assemblages in individual CSTRs, the SIMI index was used to compare algal samples collected on January 22, 1980 to samples collected during DEP additions. SIMI values were based first on all taxa present in the sample and then on the green and bluegreen algal divisions separately (see Tuchman and

Blinn 1979 for a similar treatment of algal divisions). Results of this comparison are presented in Table 9; calculated SIMI values ≥ 0.50 are arbitrarily considered to reflect similar species assemblages whereas values < 0.50 indicate dissimilar assemblages. These results should be considered tentative because many of these comparisons are based on only one sample.

Based on all observed taxa, CSTRs 3-5 maintained generally similar aufwuchs species assemblages while CSTRs 6 and 8 developed dissimilar assemblages, with an apparent trend of decreasing similarity proceeding downstream. It is noteworthy that the bluegreen algae maintained highly similar taxa in all CSTRs except CSTR 8, while the green algae developed generally dissimilar taxa in all CSTRs except CSTR 4. Further resolution of the aufwuchs algal assemblages from these two sampling periods is provided by Tables 10 and 11, summarizing the relative abundance of observed algal species comprising $>$ 1.0% of the total relative density. From these tables it is apparent that the temporal stabilities of CSTRs 3-5 are primarily dependent upon the dominance of *Phormidium sp.*, a filamentous cyanophyte that had an average relative abundance of 76.5% in CSTRs 3-5 on January 22, 1980 and 33.6% during the DEP additions. The strong dissimilarity between SIMI values for the bluegreen taxa in CSTR 8 is primarily due to the marked decrease (from 36% to 3.8%) in relative density of *Phormidium sp.* and to the large increase (from 1.0% to 54.3%) in *Chroococcus dispersus*, a coccoid cyanophyte. The generally dissimilar SIMI values observed for the green algal taxa in CSTRs 6-8 are mainly due to a temporal change in dominant *Scenedesmus* species; on January 22, 1980 *Scenedesmus dimorphus* comprised 72% of the total relative density of green algal taxa observed in these CSTRs, while during DEP additions *Scenedesmus bijuga* accounted for 44.8%.

In general, the existing data suggest that the aufwuchs assemblages in AEcoS reached fairly stable levels of biomass, metabolic activity, and similar species composition within 2 or 3 months after inoculation. This is most apparent in the nutrient enriched CSTRs (3, 6, 7, and 8); CSTRs 4 and 5 may have taken longer because of the low concentrations of nutrients reaching them. In addition, CSTRs 4, 5, 7, and 8 may have experienced a time lag in development corresponding to the distance from initial nutrient input.

Due to the high levels of nutrient loading and light intensity influencing all of the CSTRs, space may have been the ultimate factor limiting growth in the communities. With continual sloughing of attached growth, the CSTRs may have operated as chemostats, the rate of particulate wash-out equalling new growth for perhaps most of the experiment, but exceeding new growth near the end.

THE NATURE OF DIFFERENT ENVIRONMENTS IN AEcoS

One objective of the experimental manipulations in the DEP experiment was to create different chemical and biological environments for toxicant exposure in AEcoS. This section considers (1) the differences among the biotic communities in AEcoS, (2) the behavior and possible influences of phosphorus and nitrogen on these differences, and (3) the combined influence of nutrient inputs and biotic community structure on the appearance of unexpectedly high concentrations of nitrite-nitrogen in AEcoS.

Taxonomic Structure

Differences in aufwuchs algal species composition in CSTRs A3-A8 and B3-B8 during DEP additions were evaluated with the SIMI index. SIMI values were first calculated for all taxa observed in the sample and then for the green and bluegreen algal divisions separately.

Results of these comparisons are summarized in Table 8. To facilitate interpretation, comparisons that resulted in SIMI values greater than 0.50 were grouped together in a format similar to that of a multiple range test; these results are presented in Tables 9 and 10.

In general, comparisons of aufwuchs algal species composition within a channel are more similar than dissimilar and reflect the inherent difficulty in establishing and maintaining distinct algal assemblages under the regime of physical and chemical parameters used in the study. The combination of high light intensity, abundant inorganic nutrients, low buffering capacity, and relatively high water temperature resulted in selection for euryecious organisms typical of eutrophic systems. Furthermore, dissimilar SIMI values were usually attributable to large changes in relative abundance of one or two taxa; for example, in Channel B, CSTRs 3-6 were grouped separately from CSTRs 7 and 8 based on all observed taxa primarily because of differences in relative abundance of *Phormidium sp.* and *Chroococcus dispersus* (Table 11 and 12). It is likely that inherent spatial variability, sampling errors, and insufficient sample replicates also contributed to observed differences.

Calculated values of H' diversity and J evenness during DEP additions (Table 13) ranged, respectively, from 1.69 to 2.69 and from 0.47 to 0.63 in Channel A and from 1.27 to 3.14 and from 0.32 to 0.74 in Channel B. Although these indices are sensitive to the same problems discussed above, they do indicate that these systems were representative of relatively complex aquatic ecosystem. There was no apparent relationship between CSTR treatments and calculated diversity and evenness indices.

Non-Taxonomic Structure

An evaluation of the existence of structurally distinct aufwuchs assemblages in CSTRs 3-8 during DEP additions was made by performing a one-way analysis of variance on chlorophyll *a*, ATP, AFDW, TOC, and bacterial data and summarizing any significant differences with the Newman Keuls multiple range test. Results of the multiple range test are presented in Table 14.

In general, estimators of living aufwuchs material (chlorophyll *a*, ATP, and bacteria) provided greater resolution in distinguishing structurally different assemblages than did those estimators that also include detritus (AFDW and TOC). For example, chlorophyll *a* and ATP concentrations were significantly different in 4 out of 6 CSTRs while AFDW and TOC estimates were not shown to be different in any individual CSTR. Although export of particulate material was not measured in this study, it is possible that the non-significant differences observed in AFDW and TOC estimates can be partially explained by sporadic sloughing of aufwuchs material and subsequent export downstream.

Results also indicate that different nutrient regimes influenced the measured structural components of aufwuchs assemblages. CSTR 6, which received high concentrations of inorganic nutrients, was significantly different from low nutrient CSTRs 3-5 for all variables tested. CSTR 5, which received glycerin to stimulate heterotrophic activity, had unexpectedly low bacterial numbers; low inorganic nutrient concentrations apparently limited organic carbon utilization in this CSTR and subsequent downstream export resulted in the significantly highest bacterial numbers in CSTR 6.

Chlorophyll a, ATP, AFDW, TOC, and bacterial numbers collected for CSTRs 3 and 6 on the 8 major sampling dates (Table 4) were compared using the Mann-Whitney test, a nonparametric analogue of the Student t-test. This analysis tested the hypothesis that the high inorganic nutrient load into CSTR 6 would result in consistently higher biomass estimates than in low nutrient CSTR 3. Results indicate that for all biomass parameters tested, significantly higher ($p = 0.05$) biomass was observed in CSTR 6. Although not unexpected, these results substantiate the differences observed between these two CSTRs during DEP additions and support the assumption that structurally different biological communities existed in AEcoS.

Trace Metal Composition

Trace metal composition of the aufwuchs samples (Table 17) were within the ranges found in studies on tropical marine algae (Sivalingham 1978), stream aufwuchs (Friant and Koerner 1981), and lentic phytoplankton (Goldman et al. 1972). There is a wide range of algal metal concentrations in the above studies because it is generally believed that algae take up metals in proportion to the ambient environmental concentrations (Friant and Koerner 1981, Wong 1980).

Concentrations of Cu, Zn, and Mg per gram dry weight were higher in aufwuchs samples taken from CSTRs 6-8 than from CSTRs 3-5 (Table 17). Manganese concentrations, however, exhibited a general decrease from CSTRs 3-5 to CSTRs 6-8.

In soils the availability of Mn and Fe (and perhaps other trace metals) depends on pH, redox potential, and organic content (Mayer and Gorham 1951). These factors are also important in aquatic systems (Hutchinson 1973) and may determine trace metal accumulation. According to Hutchinson (1973), Fe is in excess of Mn in most aquatic plants but the ratio of Mn:Fe varies, generally being below 1.0. The ratios in AEcoS aufwuchs were 0.70 ($s = 0.22$) for CSTRs 3-5 and 0.14 ($s = 0.05$) for CSTRs 6-8.

Ash percent (Table 17) ranged from 6.13 to 22.8%, and generally agrees with data from Goldman et al. (1972).

Influence of Nutrients

The importance of phosphorus in relation to aquatic ecosystem development and trophic state has been examined in some detail (Sakamoto 1966, Vollenweider 1968, Edmondson 1972, Dillon and Rigler 1974, Schindler 1977, Smith and Shapiro 1981). Current theory holds phosphorus to be the single most important limiting nutrient in many freshwater systems, and dissolved inorganic phosphorus (PO_4-P) is considered to be the most available form for plant nutrition (Chiaudani and Vighi 1974, Wetzel 1975).

The relationship between phosphorus concentration and chlorophyll a concentration has been the subject of research in both lakes and laboratory microcosms. It has been found that maximum spring or winter concentrations of phosphorus in lakes are directly proportional to summer planktonic chlorophyll a concentrations in a number of temperate lakes (Sakamoto 1966, Lund 1970, Dillon and Rigler 1974, Hickman 1980). It also has been observed that many lakes exhibit concentrations of chlorophyll a, nitrogen, and carbon proportional to phosphorus concentrations, based on mean annual values (Schindler 1978). The proportional relationship of phosphorus to chlorophyll a has also been demonstrated in both closed microcosms and simple flow-through ecosystems (Fraleigh 1978). This section examines the relative importance of phosphorus inputs in relation to aufwuchs standing crop in AEcoS.

Mean total dissolved phosphorus input concentrations and aufwuchs chlorophyll a concentrations after 4 weeks in each CSTR (Table 19) were used to develop the log-log linear regressions shown in Figure 72. Channels A and B are presented separately to demonstrate the replicability of the duplicate CSTRs. The linear regression for both channels combined is also presented to give the overall relationship of phosphorus to chlorophyll a in AEcoS. The linear regression equations are

1. Channel A: $\log_{10}(\text{chl } \underline{a}) = 0.973 \log_{10} (P) + 2.512$ ($r = 0.87$, $p < 0.001$)
2. Channel B: $\log_{10}(\text{chl } \underline{a}) = 0.855 \log_{10} (P) + 2.566$ ($r = 0.91$, $p < 0.001$)
3. Channel A and B: $\log_{10}(\text{chl } \underline{a}) = 0.905 \log_{10} (P) + 2.548$ ($r = 0.88$, $p < 0.001$)

Literature values for correlation coefficients of linear regressions of total phosphorus versus chlorophyll a from planktonic investigations include $r = 0.67$ (Hickman 1980), $r = 0.86$ (Schindler 1977), and $r = 0.98$ (Dillon and Rigler 1974); r values from the present study similarly reflect a strong correlation.

In an effort to demonstrate an even greater correlation between phosphorus input and aufwuchs chlorophyll a concentrations, two factors that might cause deviations from linearity in the relationship were considered. These were limitations of nitrogen and light to the community. Dillon and Rigler (1974) used the data of Sakamoto (1966) to demonstrate that the greatest deviations from the regression line of phosphorus versus chlorophyll a occurred in lakes with low N:P ratios. The N:P input ratios in AEcoS, based on total dissolved nitrogen and total dissolved phosphorus concentrations, varied from 14.8 to 65.3 during the period of study, none falling below the N:P ratio of 12 considered by Dillon and Rigler (1974) to indicate nitrogen limitation. Hickman (1980) observed that, as lakes progress from oligotrophy to eutrophy, decreases in light penetration and euphotic zone depth cause the standing crop of phytoplankton to approach its theoretical maximum as a result of self-shading. He suggested that a curvilinear relationship between phosphorus and planktonic chlorophyll a is more ecologically appropriate than a linear relationship and derived the equation:

$$\log_{10} (\text{chl } \underline{a}) = 3.27 \log_{10} (\log_{10}[P]) + 0.542 \qquad (4)$$

where $r = 0.842$, $p < 0.001$. Data from Channels A and B combined (Table 19) were used to determine whether such a relationship existed in AEcoS. The following curvilinear equation was derived

$$\log_{10}(\text{chl } \underline{a}) = 2.439 \log_{10} (\log_{10}[TDP]) + 3.583 \qquad (5)$$

where $r = 0.91$, $p < 0.001$ (Figure 73). Therefore, the curvilinear relationship ($r = 0.91$) shows a stronger correlation than the linear relationship ($r = 0.88$).

In addition to self-shading, aufwuchs communities are affected by spatial limitations not found in planktonic communities. Plankton are usually surrounded by available gases and nutrients as they change position in the water column. In contrast, periphyton are limited to substrates where diffusion of gases and nutrients is restricted; even if biomass continues to accumulate, only the upper layer of the periphytic mat may remain photosynthetically active (McIntire and Phinney 1964, Sumner and Fisher 1979). As Figure 73 illustrates, chlorophyll a concentrations began to level off despite increasing total dissolved phosphorus inputs. Therefore, the curvilinear relationship does seem more ecologically appropriate for these aufwuchs communities.

Highly significant correlations among aufwuchs chlorophyll a, ash-free dry weight, ATP, and TOC (Table 16) suggest that similar relationships exist between phosphorus input and other aspects of community structure. Thus, manipulation of phosphorus inputs appears to be an effective means of creating biotically distinct environments in AEcoS, particularly with respect to non-taxanomic structure.

In this respect, the relative importance of nitrogen appeared to be less than that of phosphorus. Inorganic nitrogen (NH_4NO_3) and phosphorus ($NaH\ PO_4 \cdot 7\ H_2O$) were introduced at N:P weight ratios of 14.76 - 14.82 (CSTR 3) and 16.77 - 16.93 (CSTR 6, including inputs from CSTR 5) (Table 20). These values fall within the range considered to be optimal for algal growth (Sakamoto 1966, Lund 1970, Schindler 1977, Rhee 1978). Inorganic N:P ratios in the outflowing water (Table 20, Figures 74 and 75) were generally higher than input values in both CSTRs, suggesting that phosphorus was taken up to a larger degree than was nitrogen. Therefore, downstream CSTRs (especially CSTRs 7 and 8) may have received an excess of nitrogen relative to phosphorus, but with no proportional increase in periphytic growth. The absence of nitrogen fixing blue-green algae from all CSTRs (Tables 10 and 11) also suggests that nitrogen was present in abundance throughout the experiment. Thus, phosphorus availability probably controlled growth of the communities.

Nitrite-Nitrogen Accumulation in AEcoS

The accumulation of dissolved nitrite-nitrogen in CSTRs 6-8 during the first half of the DEP experiment (Figures 21-23) was unexpected because of the absence of significant amounts of nitrite in most aerobic natural systems. Nitrogen in the form of nitrite was not introduced into AEcoS and was therefore the product of chemical or biological activity. Nitrite formation can result from (1) oxidation of ammonia, (2) reduction of nitrate, and/or (3) extracellular release of nitrite by phytoplankton (Mortonson and Brooks 1980).

Nitrification involves oxidation of ammonia to nitrite and then to nitrate by the bacteria *Nitrosomonas* and *Nitrobacter*, respectively, although other autotrophic and heterotrophic organisms also may participate (Painter 1970). This process usually proceeds quickly from ammonia to nitrate, but inhibition of the oxidation of nitrite to nitrate can allow nitrite to accumulate in the system. Anthonisen et al. (1976) found that *Nitrobacter* activity was inhibited by high concentrations of free ammonia (NH_3) and free nitrous acid (HNO_2) in water treatment systems. Temperature, pH, and concentrations of nitrite and total ammonia controlled the concentrations of the two inhibitory agents, free ammonia affecting the organisms at high pH and free nitrous acid at low pH. Although Anthonisen et al. (1976) worked with much higher concentrations of nutrients than those in AEcoS, inhibitory effects might also be expected at lower concentrations. The constant input of ammonia coupled with low alkalinity and high pH observed in AEcoS during the period of high nitrite concentrations (pH $>$ 9 for p.m. samples) could potentially supply free ammonia and thus inhibit *Nitrobacter* activity. Brezonik (1973) notes that a well buffered medium is necessary to avoid high pH and ammonia inhibition effects.

In denitrification, nitrate is reduced to nitrite and then to ammonia by phytoplankton, macrophytes and some bacteria, such as *Eschericia coli*, *Azotobacter*, and *Lactobacillus*. Other bacteria, including *Micrococcus*, *Pseudomonas*, and *Denitrobacillus* convert nitrate to nitrite, ammonia, nitrous oxide, or dinitrogen (Painter 1970). Since denitrification proceeds at low oxygen concentrations, such conditions in AEcoS during morning sampling (Figure 51-53) could have encouraged this process. In addition, thick periphyton mats could have provided anaerobic microzones suitable for denitrifying organisms. Comparison of the nitrite and nitrate concentration graphs of CSTRs 6-8 (Figures 18-23) reveals an inverse relationship between the two chemical species. This indicates that nitrite accumulation could have been the result of nitrate reduction.

Excretion of nitrite by phytoplankton has been studied extensively in marine environments (Vaccaro and Ryther 1960, Hattori and Wada 1971, Vorturiez and Herbland 1978) and more recently in fresh water systems (Ohmori 1978, Mortonson and Brooks 1980). Ohmori (1978) correlated excretion of nitrite by *Oscillatoria rubescens* with nitrate uptake during the initial growth phase of cultures. Nitrite accumulation was not affected by ammonia concentration or by the presence of bacteria. Nitrite excretion was apparently due to initially high levels of nitrate-reduction activity. Later, nitrite-reduction activity increased and the cells assimilated both nitrate and excess nitrite. Nitrite concentrations in Ohmori's cultures reached maximum values in 45 days and declined to undetectable levels by 70 days. These findings resemble the pattern of nitrite accumulation in CSTRs 6-8 and might explain why nitrite accumulated significantly only during the first half of the experiment. Although O. rubescens was not observed, the presence of other species of Oscillatoria was noted (Tables 10 and 11). Ohmori and Hattori (1970) have also observed nitrite accumulation due to Anabaena cylindrica.

Thus, three processes may have contributed to the accumulation of nitrite in CSTRs 6-8: (1) inhibition of nitrification, (2) stimulation of denitrification, and (3) algal excretion of nitrite. These processes may have been promoted by a combination of high light intensity, nutrient enrichment, and low alkalinity which resulted in frequent occurence of high pH and low dissolved oxygen concentrations, and in the presence of potentially nitrite-excreting algae. The absence of measurable concentrations of nitrite under lower nutrient conditions and less extreme variations of pH and DO (i.e., CSTRs 3-5) but the presence of similar algal assemblages suggests that processes (1) and (2) may have been more important than process (3) in CSTRs 6-8.

REPLICABILITY AND DIEL PATTERNS

Chemical Data

In order to evaluate replicability of chemical variables between Channels A and B of AEcoS and to compare morning and midday sampling, the nutrient data were divided into four time blocks.

a. November 27, 1980 to January 20, 1980 (early succession)
b. January 21, 1980 to May 20, 1980
c. May 21, 1980 to July 20, 1980 (acid/base addition)
d. July 21, 1980 to December 5, 1981 (DEP study)

Replicability of A and B was tested first by calculating means of A and B for each time block and then calculating means for morning samples and midday samples (lumping A and B) for each of the time blocks, finally performing t-tests on the comparisons. The nutrients tested in this manner were NO_3-N, NH_3-N, and PO_4-P in CSTRs 3 and 6.

The results in Table 21 show that for most of the experimental period, there were no differences in NO_3-N between A and B for either CSTR. The only differences were found in the last time block in CSTR 6 between Channels A and B, but it is not possible to conclude if this difference might have continued. There were two time blocks in CSTR 3 that showed significant differences in NO_3-N concentrations between sampling times, but these comparisons involve values near the detection limit of the instrumentation and may not have reflected a real difference.

There were no significant differences in NH_3-N levels in Channels A and B for either CSTR over the entire experiment. However, there were significant differences in NH_3-N levels between the two sampling times:

NH_3-N was usually lower at midday in both CSTRs 3 and 6, probably due to biotic uptake during the light cycle.

There were no significant differences in PO_4-P concentrations between CSTRs 3A and 3B except in the third time block, while CSTR 6A and 6B showed significant differences in the first and fourth time blocks. CSTR 3 morning versus midday samples showed significant differences in PO_4-P concentrations in the first two time blocks, while CSTR 6 morning versus midday samples showed significant differences in the first and forth time blocks.

In general, Channels A and B were indistinguishable with respect to nutrient concentrations for most of the experiment. The few differences that were observed were not attributable to experimenal manipulations, except perhaps effects of acid addition on PO_4-P concentrations in CSTR 3. Relatively greater PO_4-P uptake early in community development in CSTR 6A and 6B and diverging NO_3-N concentrations in the same CSTRs at the end of the experiment may reflect random variation. As expected, diel cycles of nutrient uptake and release were apparent, especially for NH_3-N. Toetz (1976) observed diel periodicity in concentrations of NO_3-N and NH_3-N in the epilimmion of a reservoir.

Aufwuchs Biomass and Species Composition

Estimates of aufwuchs chlorophyll a, ATP, AFDW, TOC, and bacterial numbers from Channels A and B during DEP additions were compared using the Student's t-test (Table 22). Results indicate that CSTRs receiving direct inputs of inorganic nutrients (CSTRs 3 and 6) had the most consistent replicability, while CSTR 8 had the poorest. Of the 5 parameters evaluated, AFDW was the most replicable with no significant differences, and bacterial estimates were least replicable with 5 significant differences.

Comparisons of aufwuchs algal species composition in Channels A and B via the SIMI index indicate that bluegreen algae were generally similar in any given CSTR comparison while green algae were generally dissimilar (Table 23). Bluegreen algal similarities are primarily due to the ubiquitous presence of *Phormidium* sp. and the occurrence of *Chroococcus dispersus* in CSTRs 7 and 8, while green algal dissimilarities in CSTRs 6-8 result from the high average relative density (46.4%) of *Scenedesmus bijuga* in Channel A and the absence of this organism in Channel B.

SECTION 8

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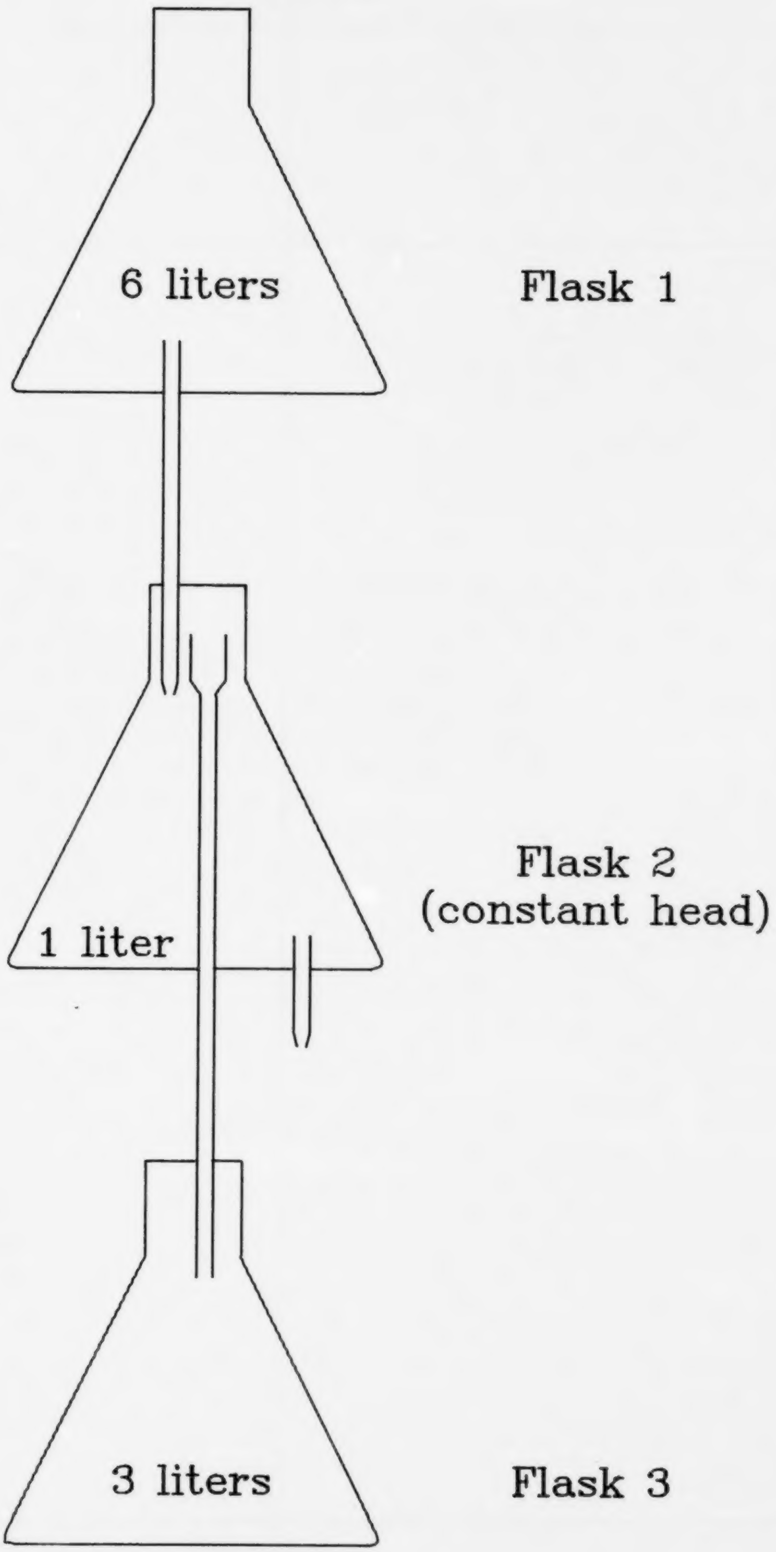
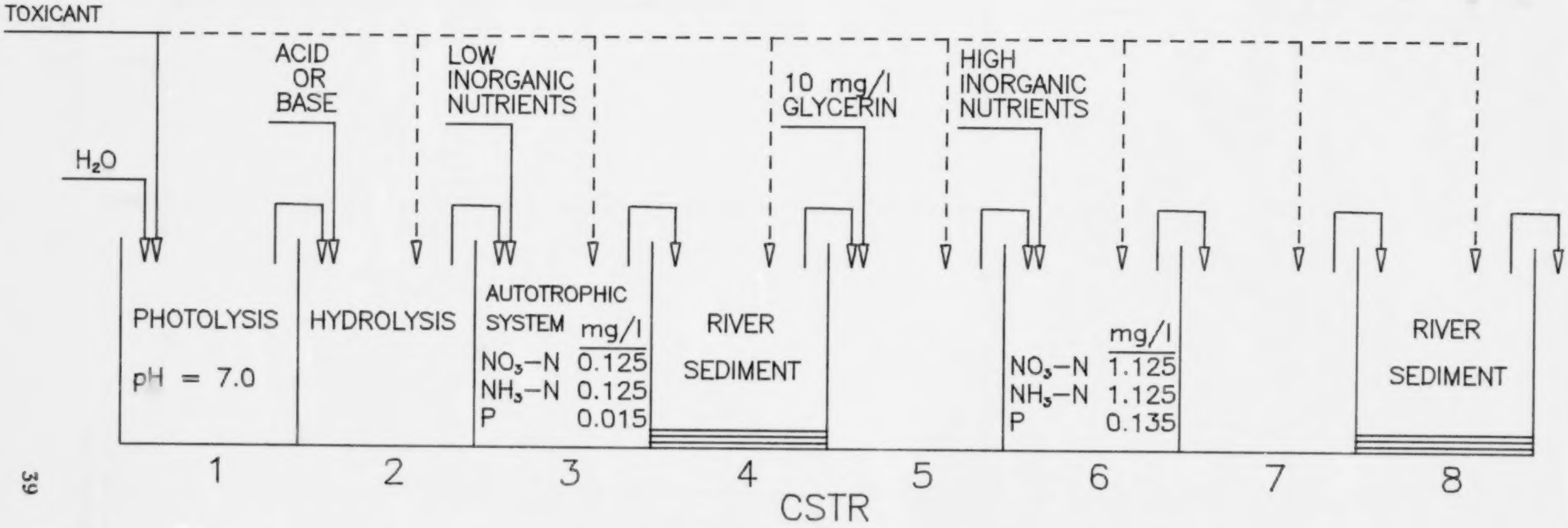

6 liters
Flask 1
1 liter
Flask 2
(constant head)
3 liters
Flask 3

Figure 1. Toxicant Feed System


TOXICANT
H₂O
ACID OR BASE
LOW INORGANIC NUTRIENTS
10 mg/l GLYCERIN
HIGH INORGANIC NUTRIENTS
PHOTOLYSIS
pH = 7.0
HYDROLYSIS
AUTOTROPHIC SYSTEM
mg/l
NO₃–N 0.125
NH₃–N 0.125
P 0.015
RIVER SEDIMENT
mg/l
NO₃–N 1.125
NH₃–N 1.125
P 0.135
RIVER SEDIMENT
1
2
3
4
5
6
7
8
CSTR

Conditions

H_2O Flow = 500 l/Day

CSTR Retention Time = 12 Hr

Mixing Rate = 2 RPM

H_2O Temp. = 20°C

Light = 2000 Foot Candles
(12 hr light, 12 hr dark)

Figure 2. Experimental Conditions of Each Channel

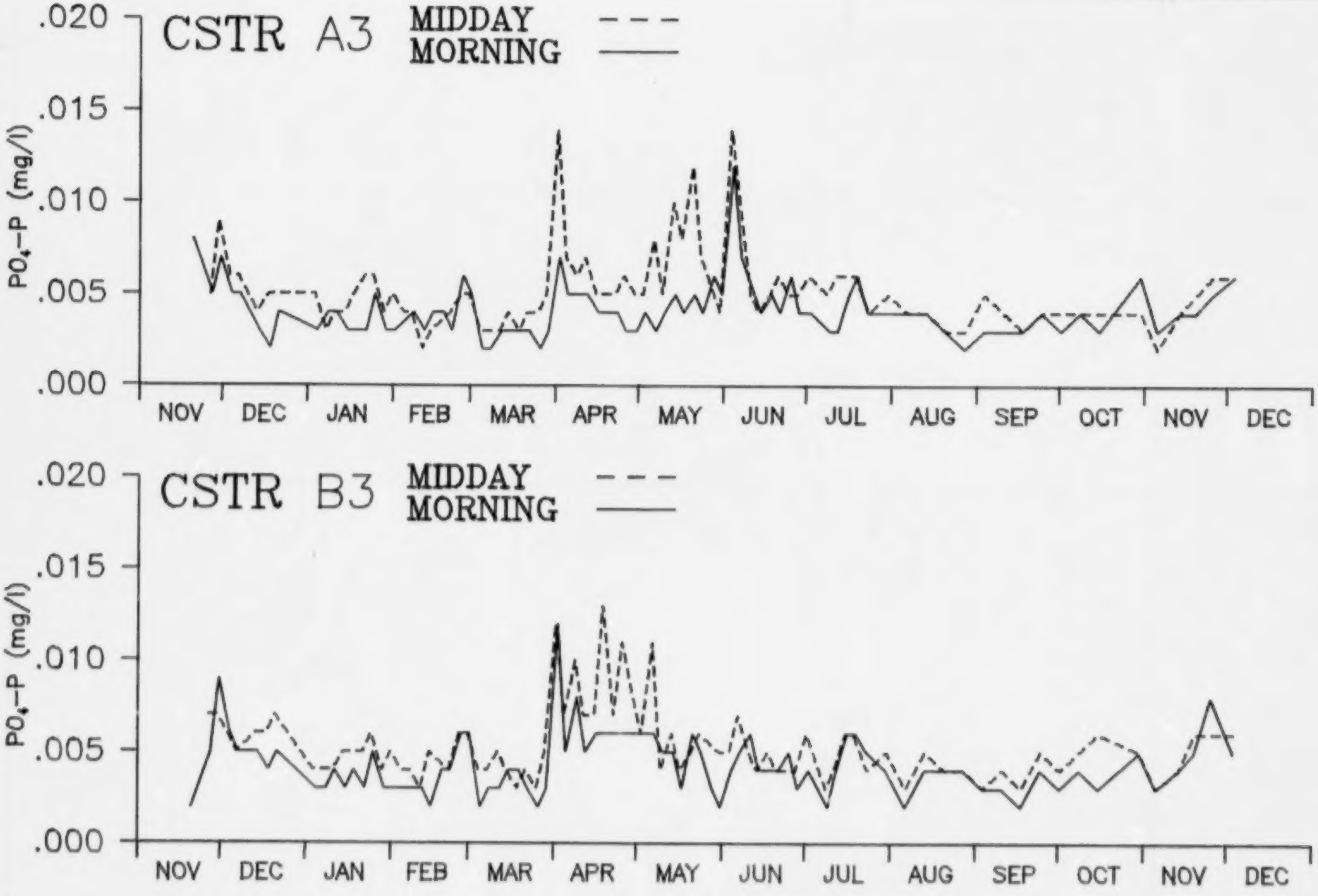


Figure 3. Orthophosphate Concentrations in CSTRs A3 and B3

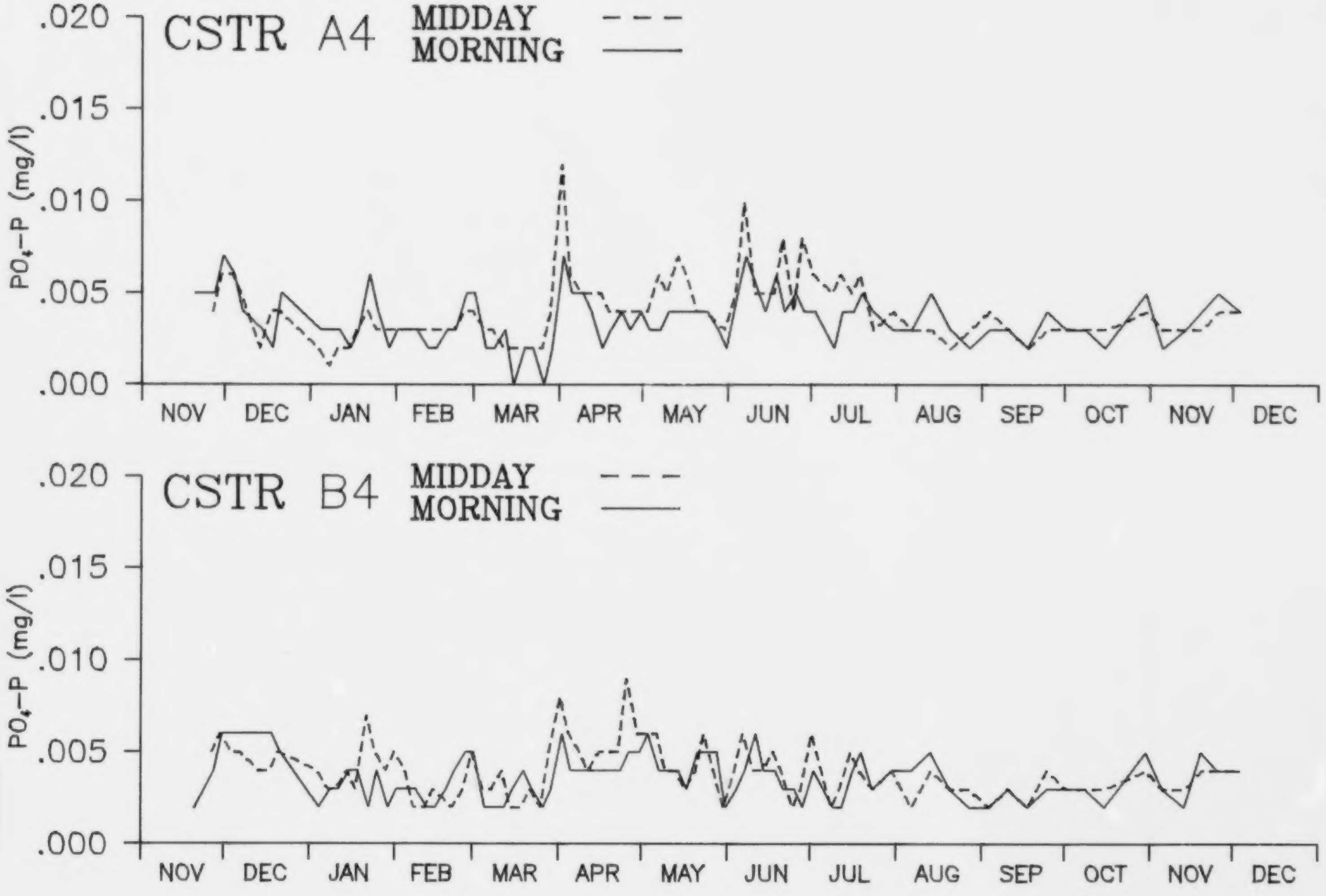


Figure 4. Orthophosphate Concentrations in CSTRs A4 and B4

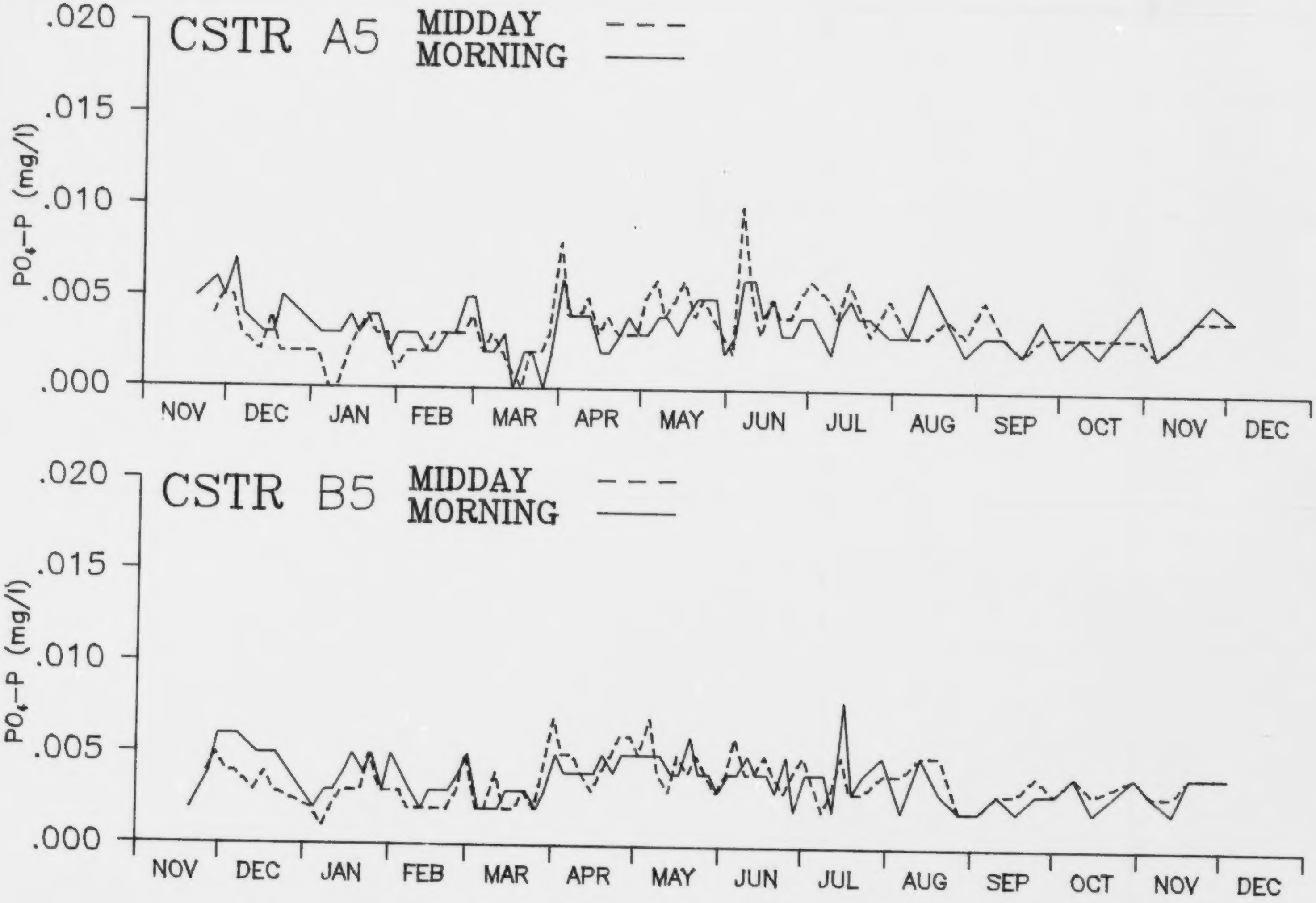


Figure 5. Orthophosphate Concentrations in CSTRs A5 and B5

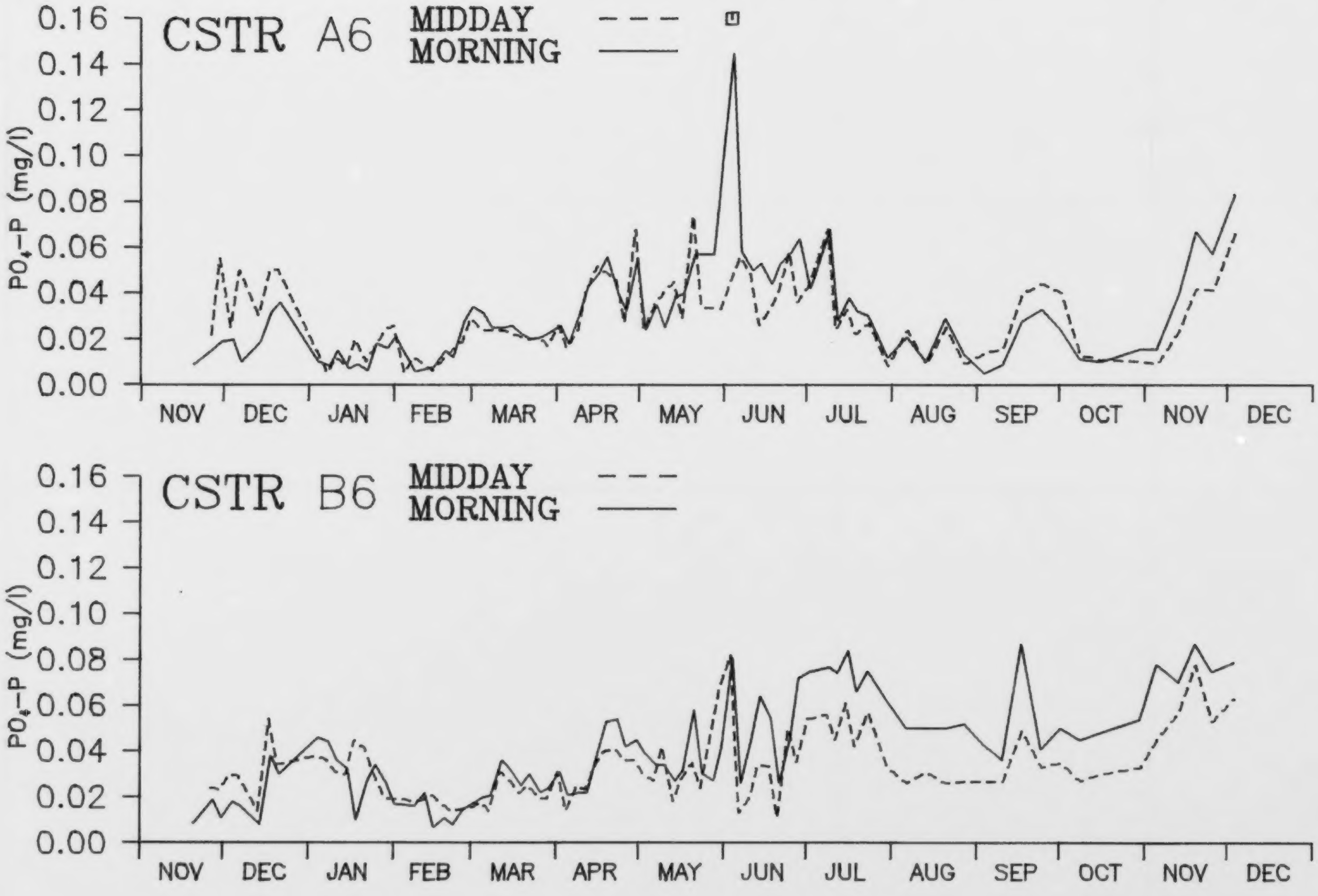


Figure 6. Orthophosphate Concentrations in CSTRs A6 and B6

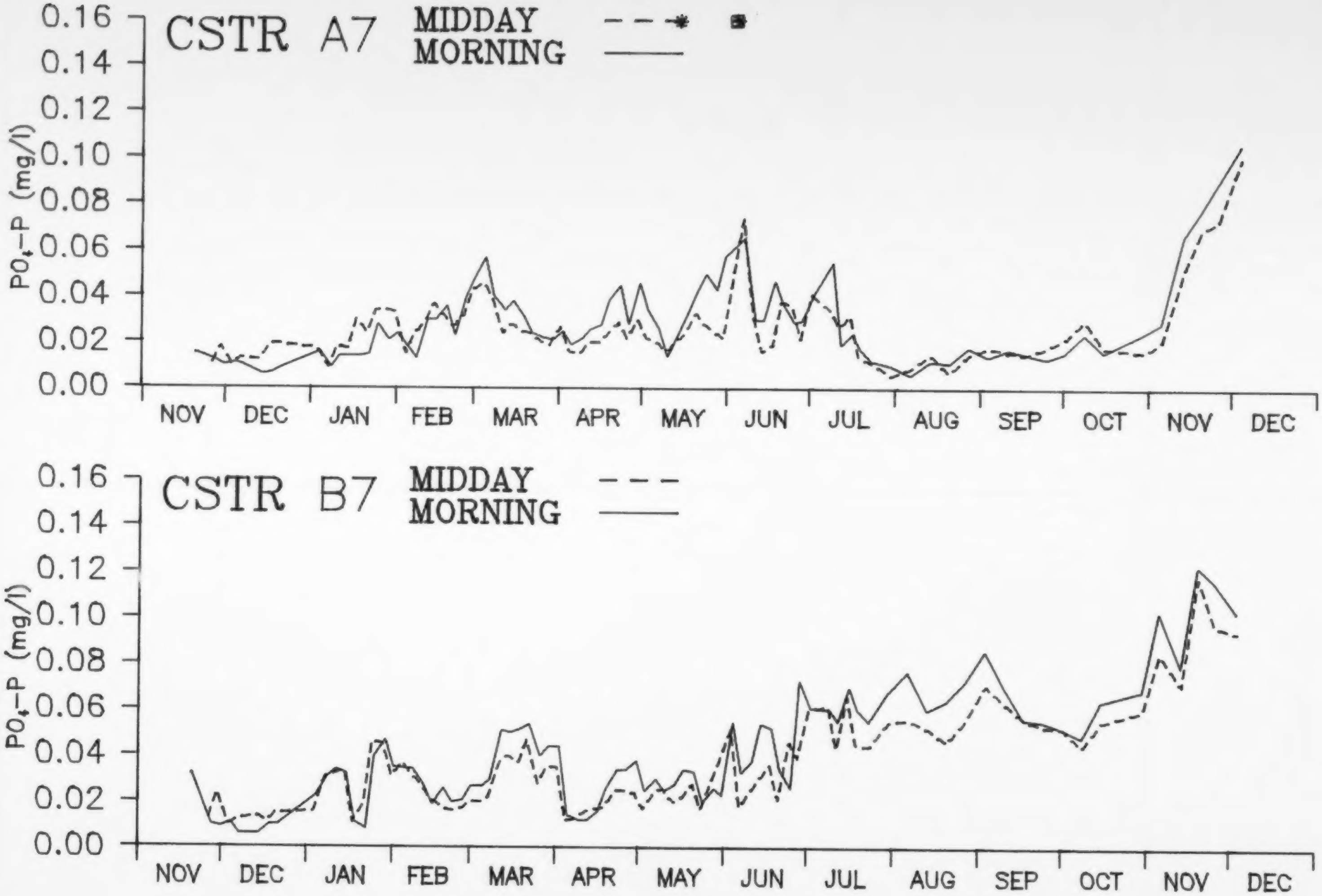


Figure 7. Orthophosphate Concentrations in CSTRs A7 and B7

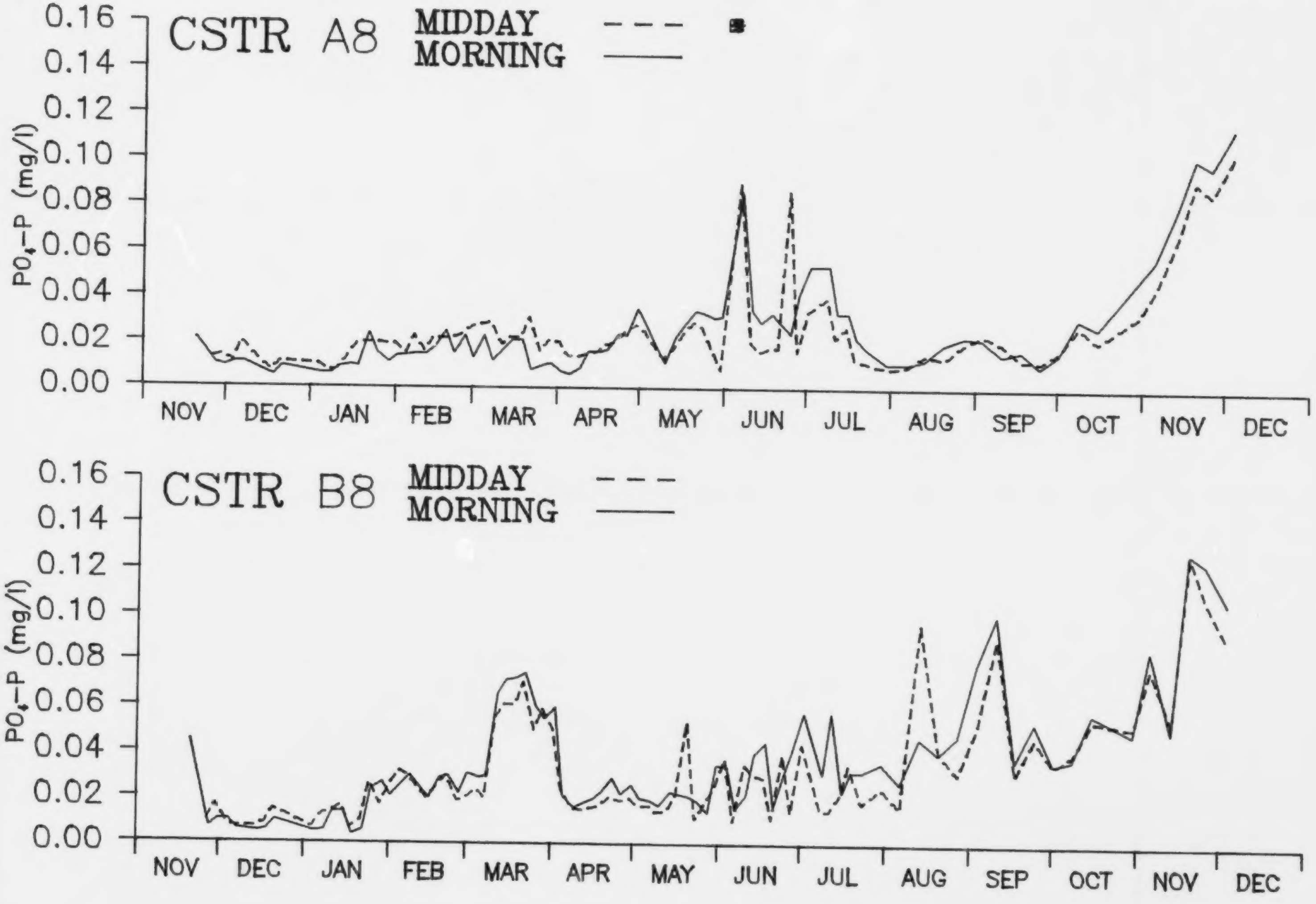


Figure 8. Orthophosphate Concentrations in CSTRs A8 and B8

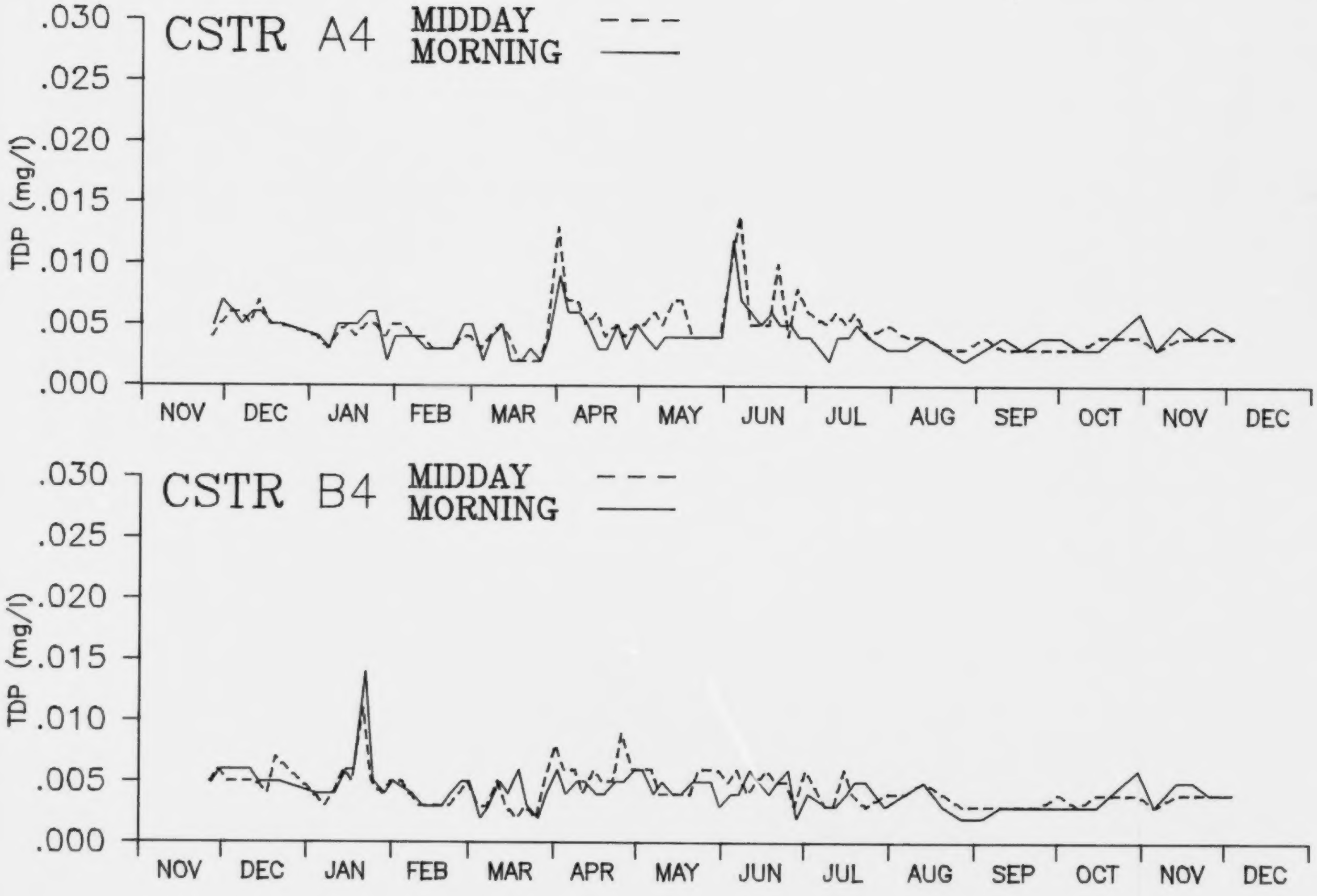


Figure 9. Total Dissolved Phosphorus Concentrations in CSTRs A3 and B3

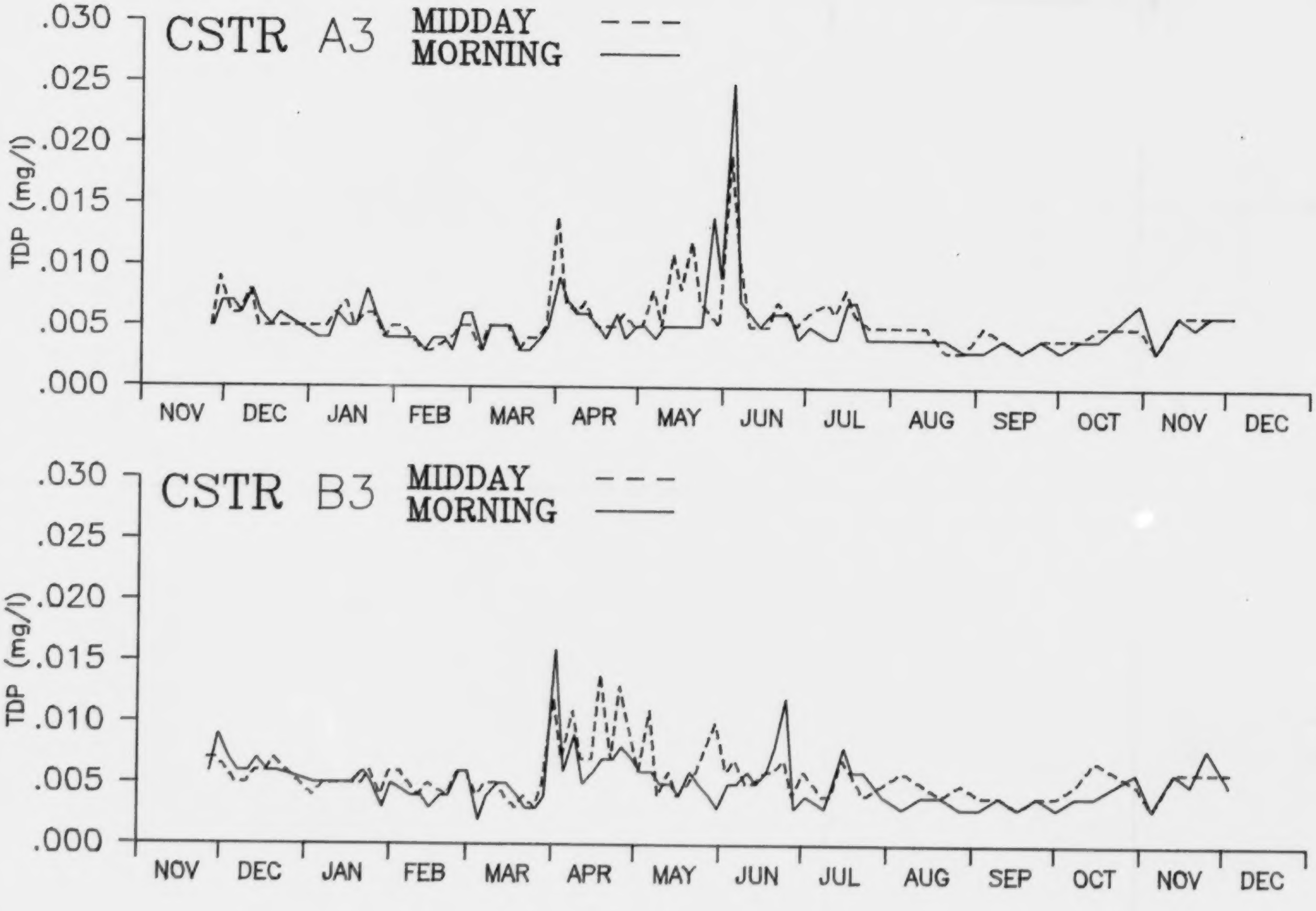


Figure 10. Total Dissolved Phosphorus Concentrations in CSTRs A4 and B4

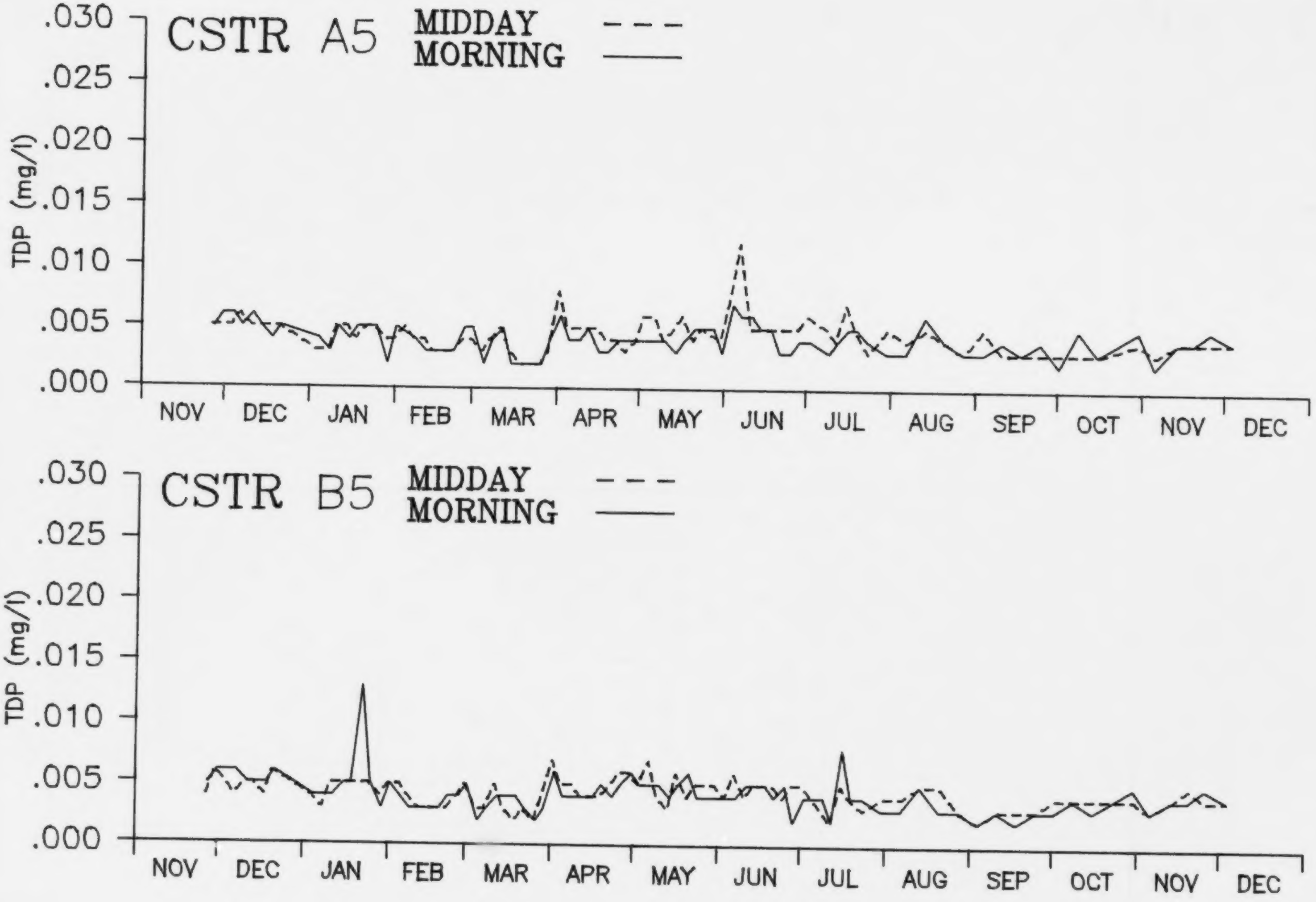


Figure 11. Total Dissolved Phosphorus Concentrations in CSTRs A5 and B5

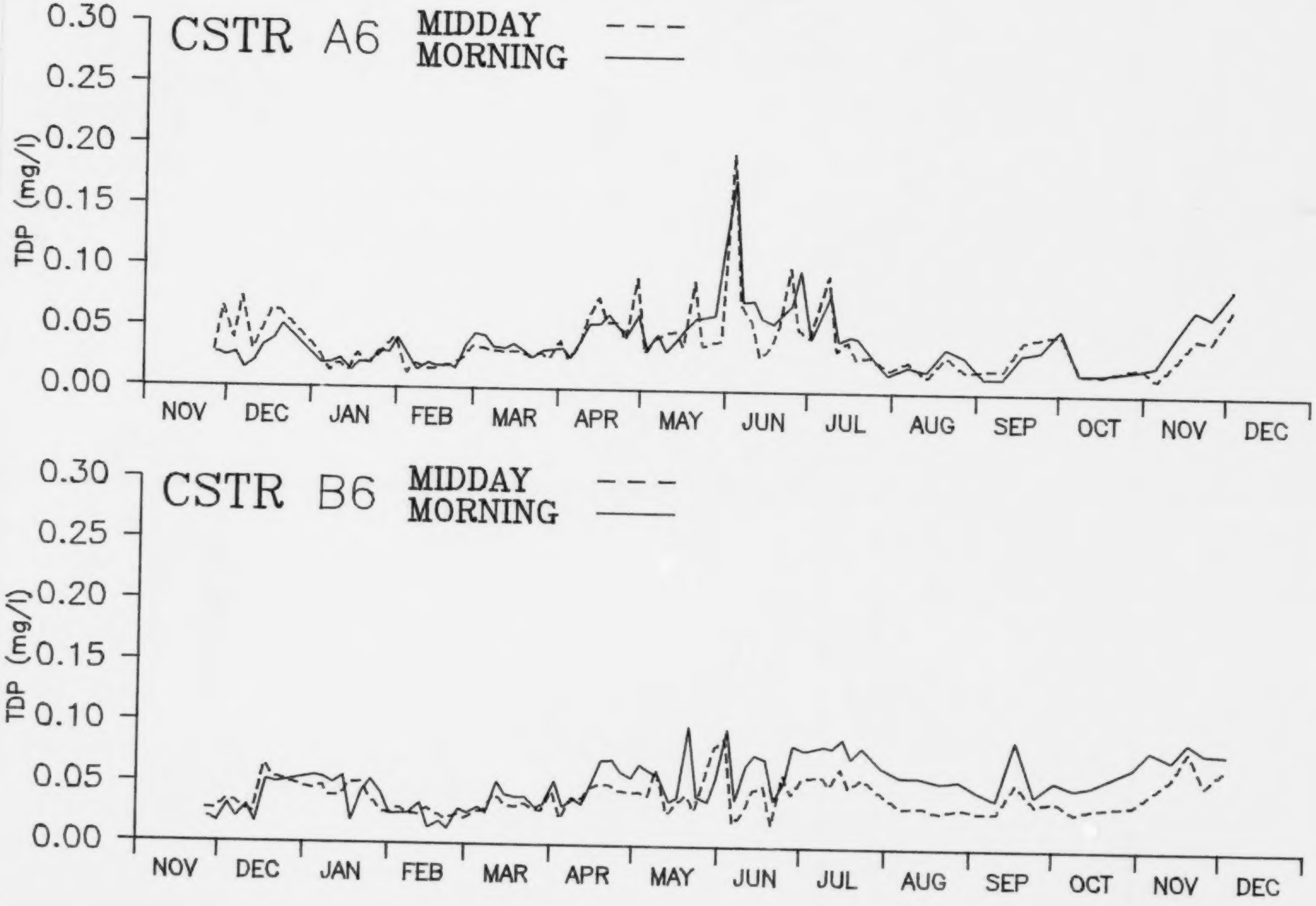


Figure 12. Total Dissolved Phosphorus Concentrations in CSTRs A6 and B6

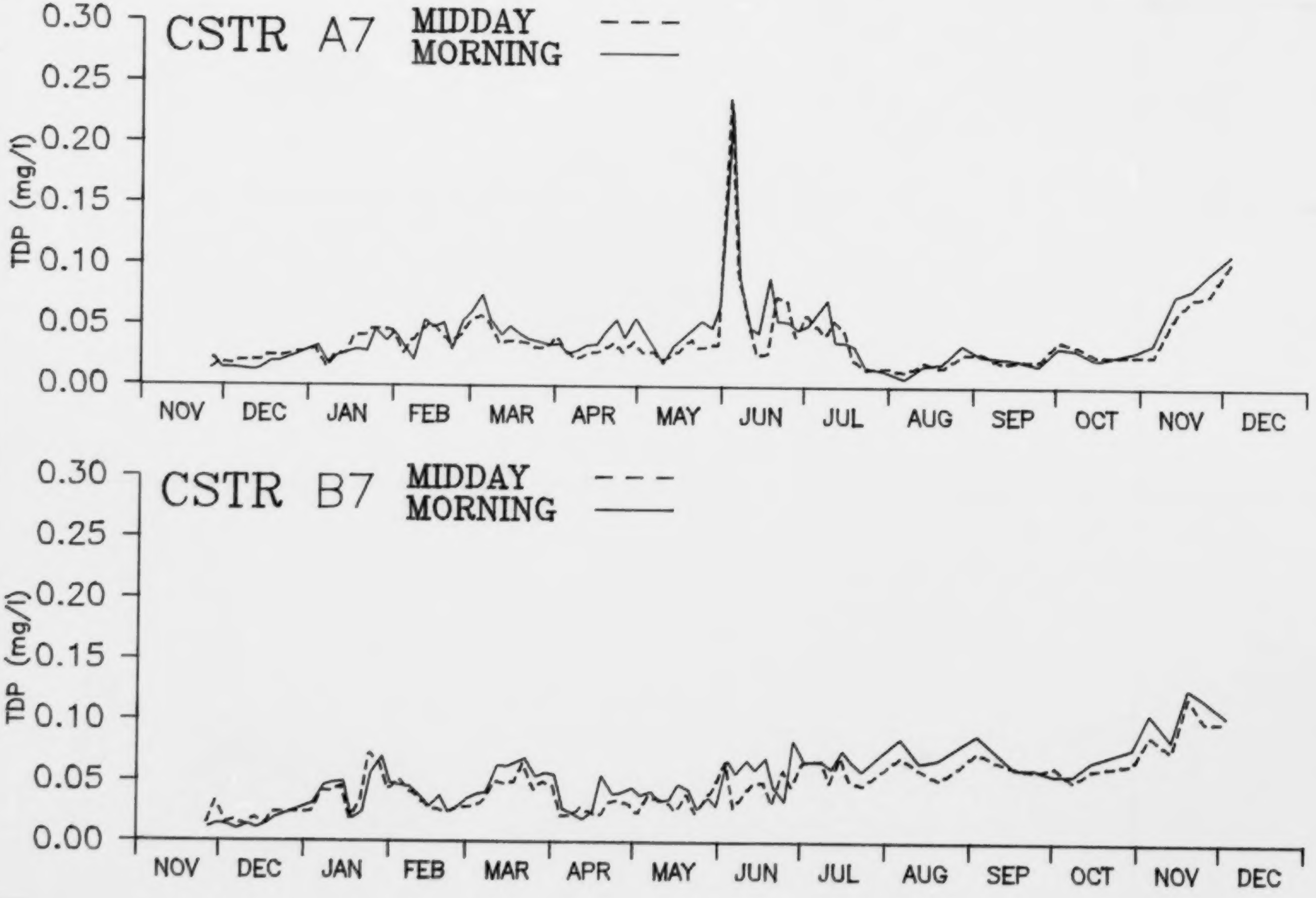


Figure 13. Total Dissolved Phosphorus Concentrations in CSTRs A7 and B7

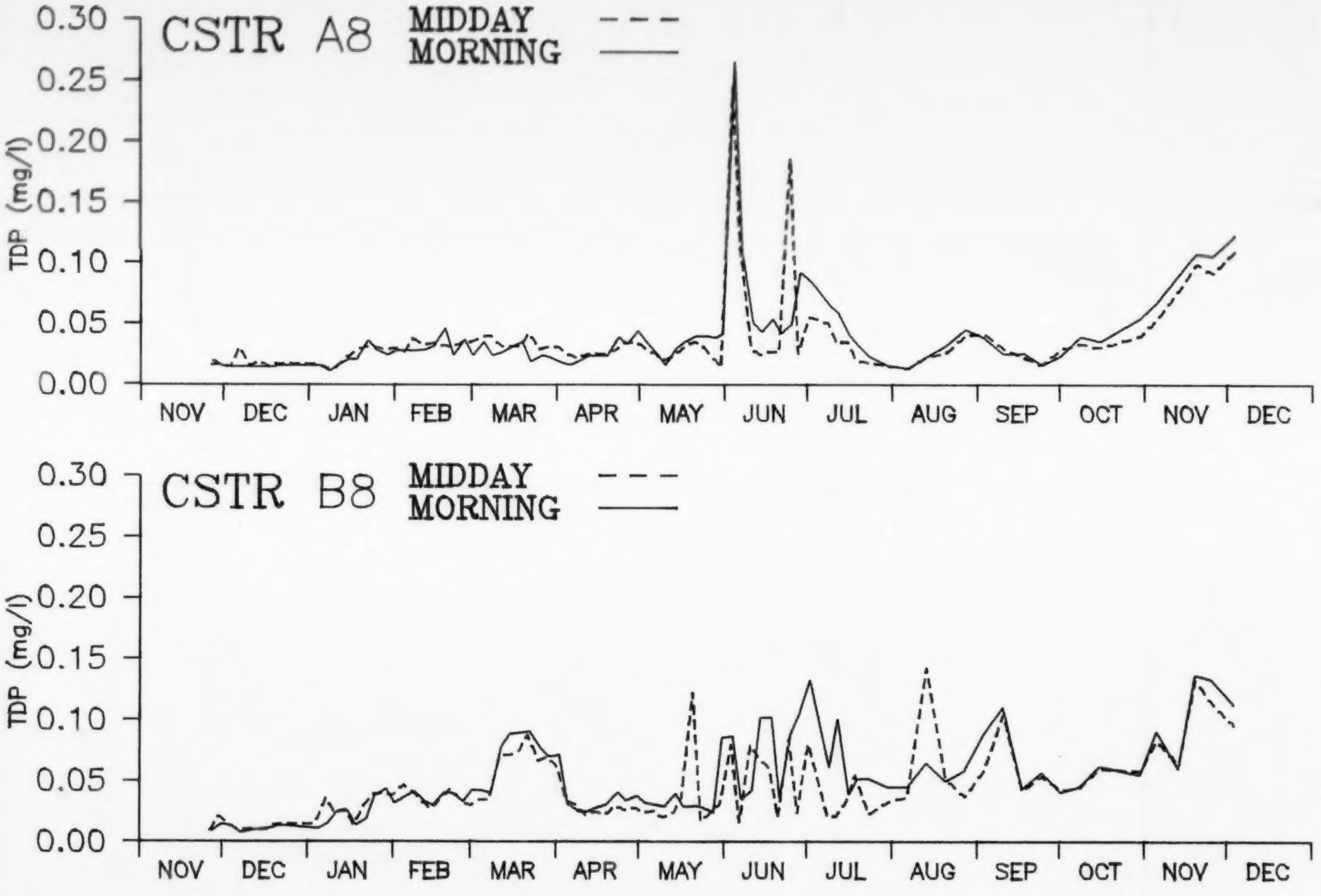


Figure 14. Total Dissolved Phosphorus Concentrations in CSTRs A8 and B8

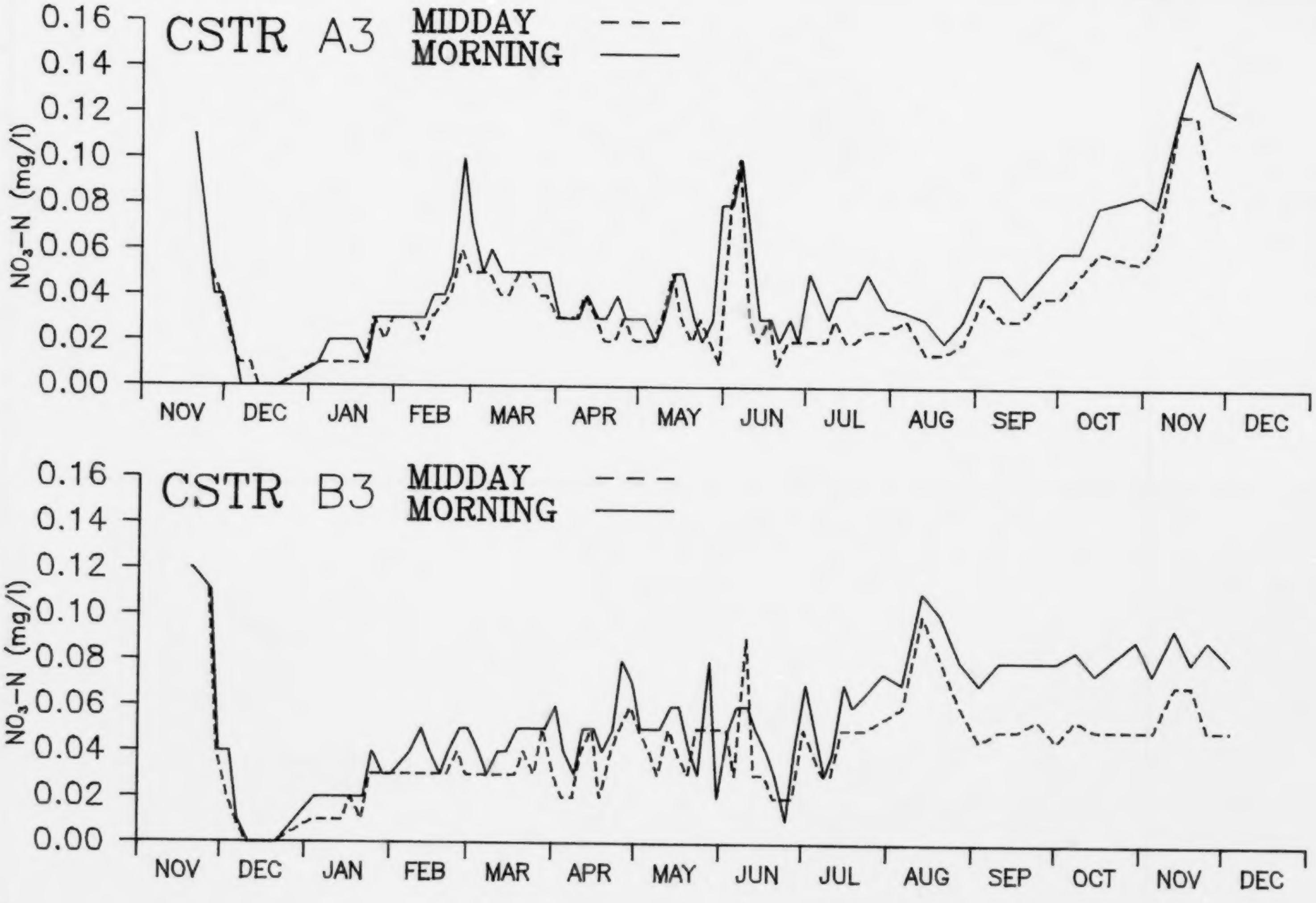


Figure 15. Nitrate-Nitrogen Concentrations in CSTRs A3 and B3

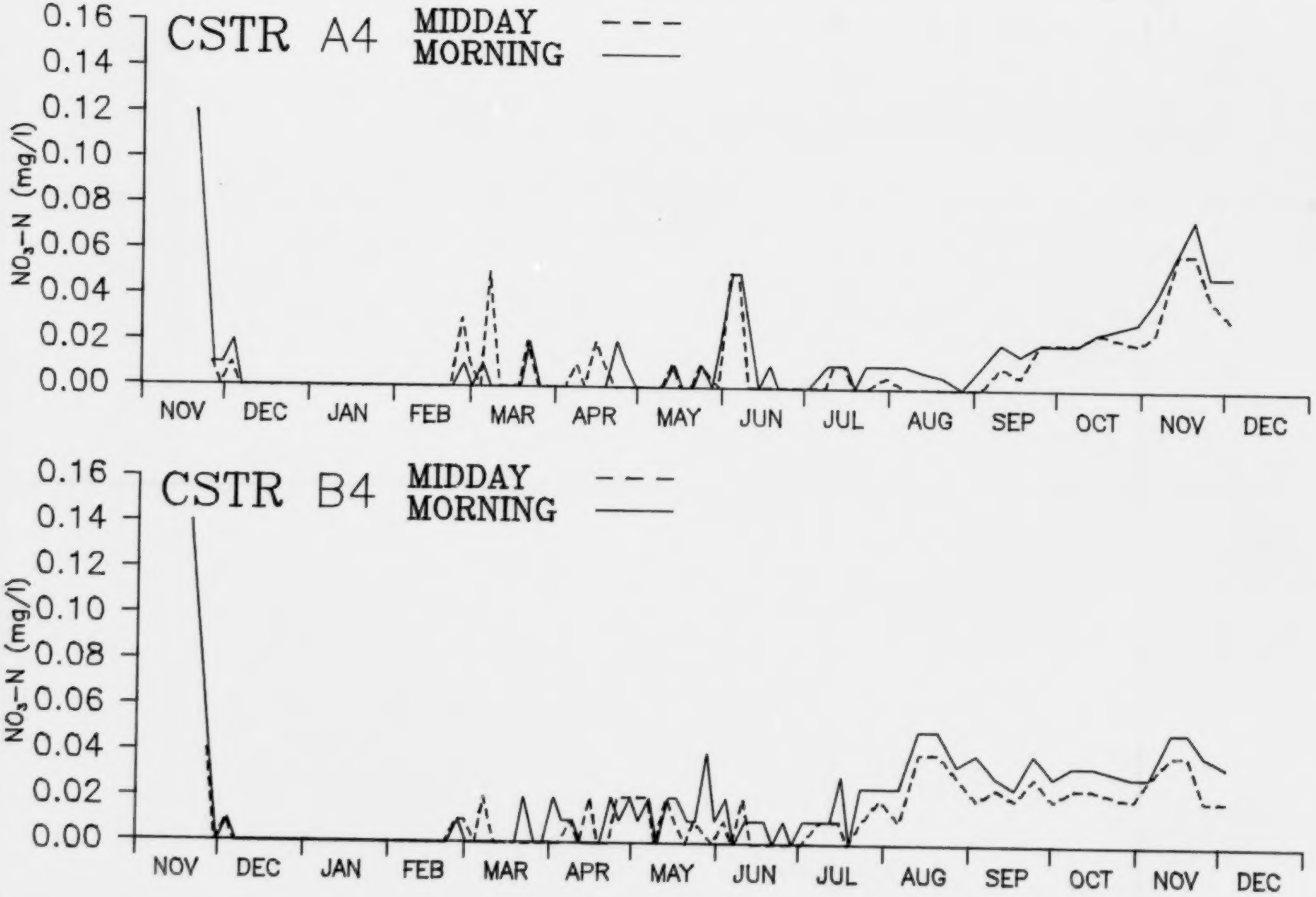


Figure 16. Nitrate-Nitrogen Concentrations in CSTRs A4 and B4

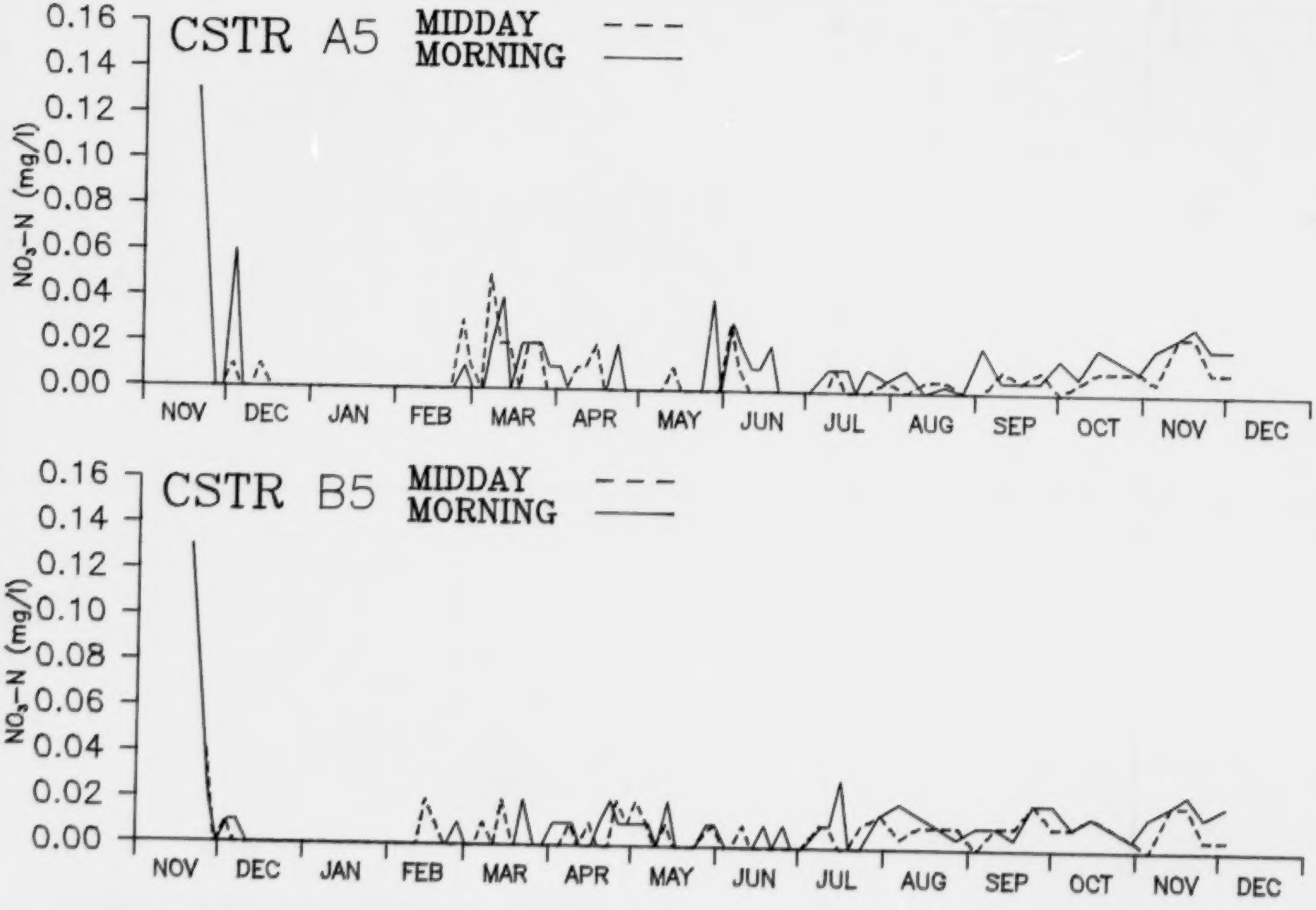


Figure 17. Nitrate-Nitrogen Concentrations in CSTRs A5 and B5

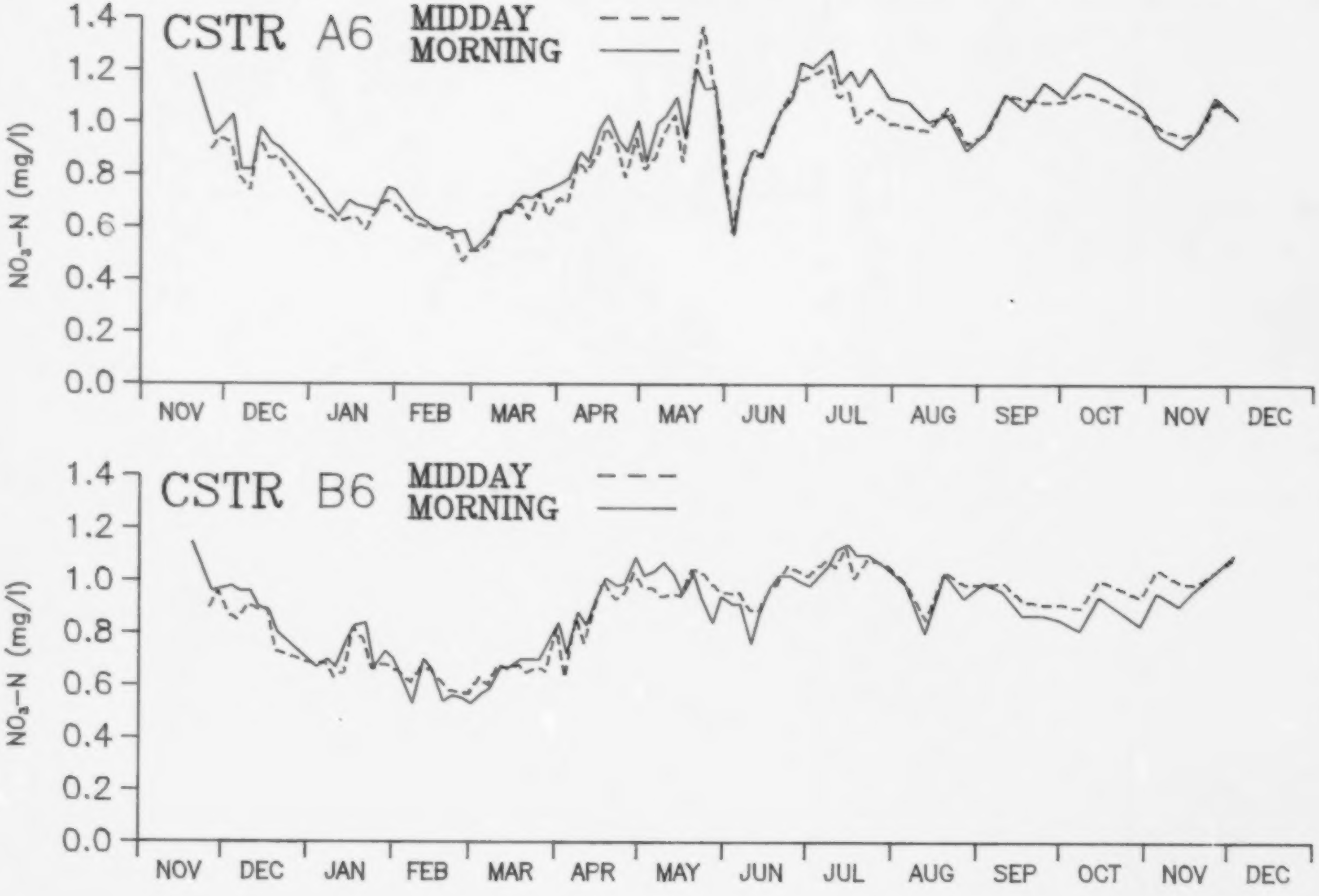


Figure 18. Nitrate-Nitrogen Concentrations in CSTRs A6 and B6

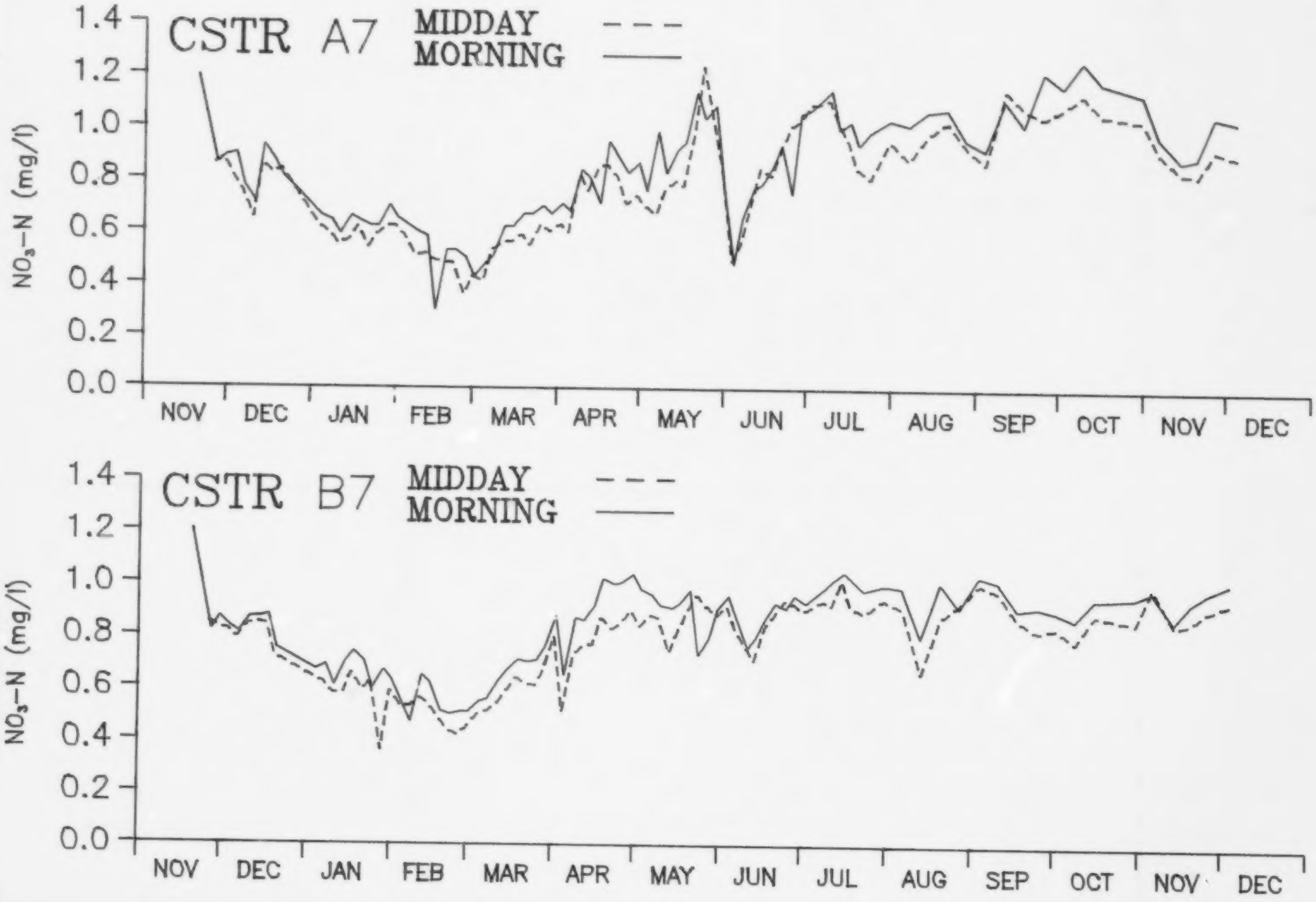


Figure 19. Nitrate-Nitrogen Concentrations in CSTRs A7 and B7

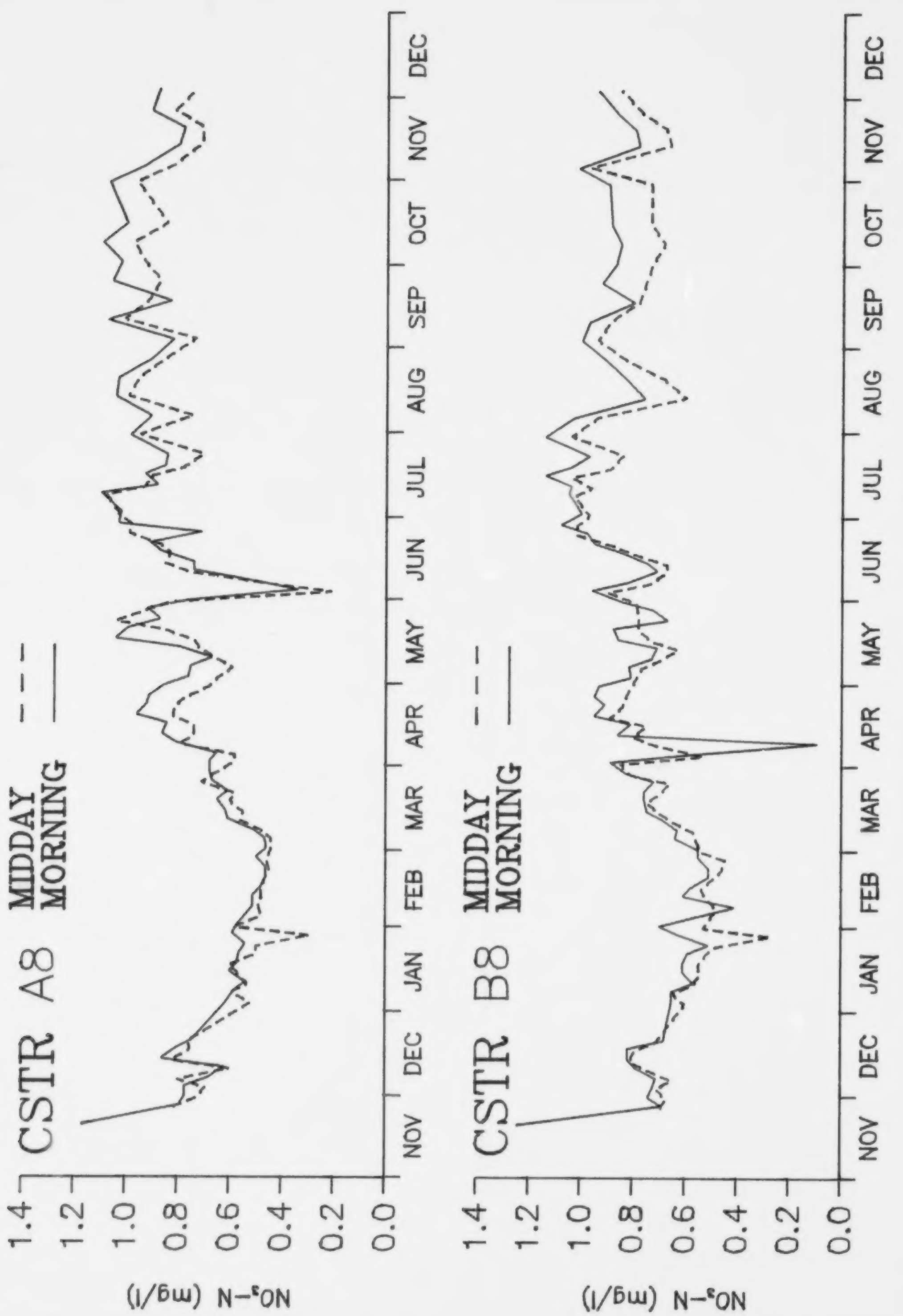


Figure 20. Nitrate-Nitrogen Concentrations in CSTRs A8 and B8

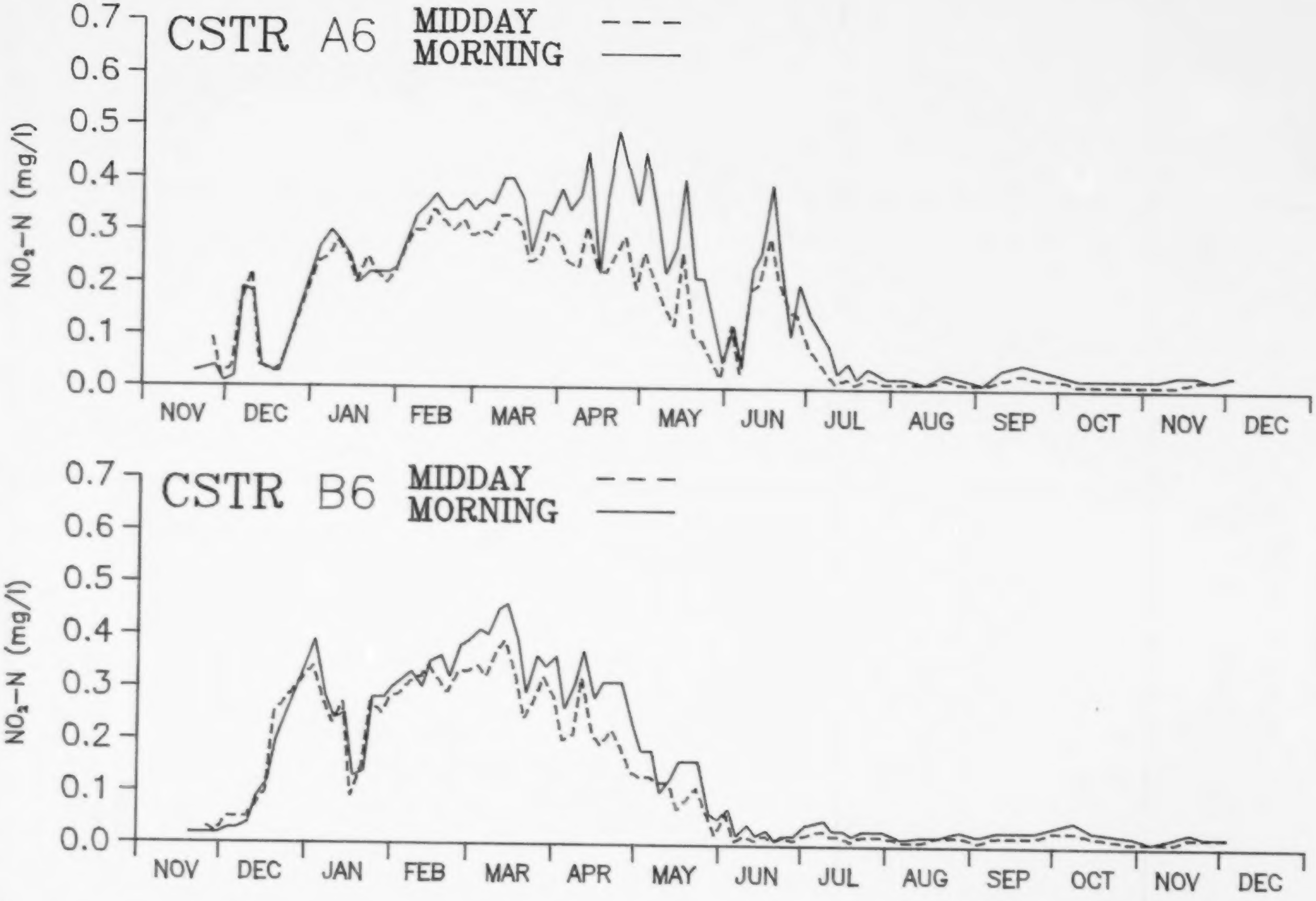


Figure 21. Nitrite-Nitrogen Concentrations in CSTRs A6 and B6

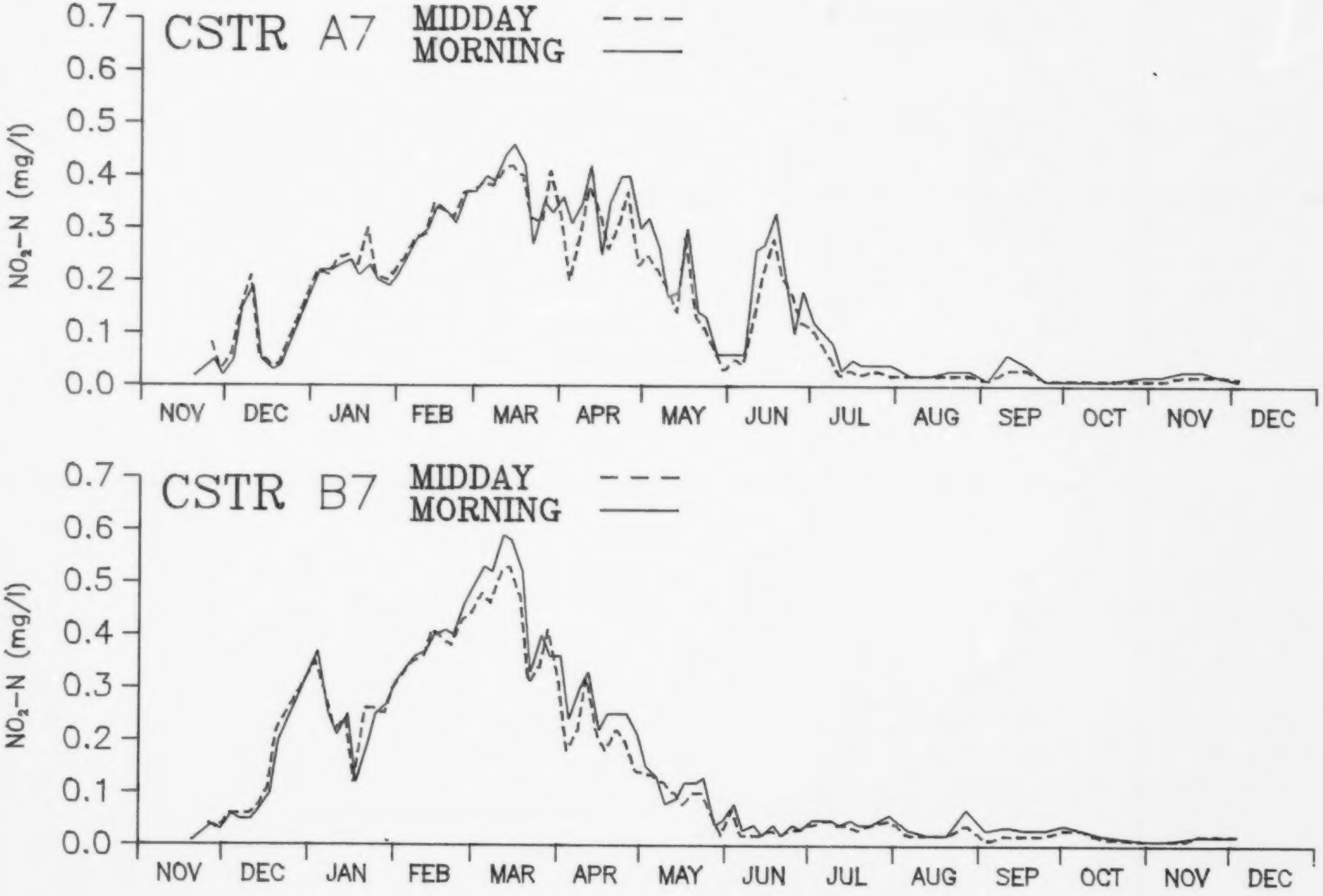


Figure 22. Nitrite-Nitrogen Concentrations in CSTRs A7 and B7

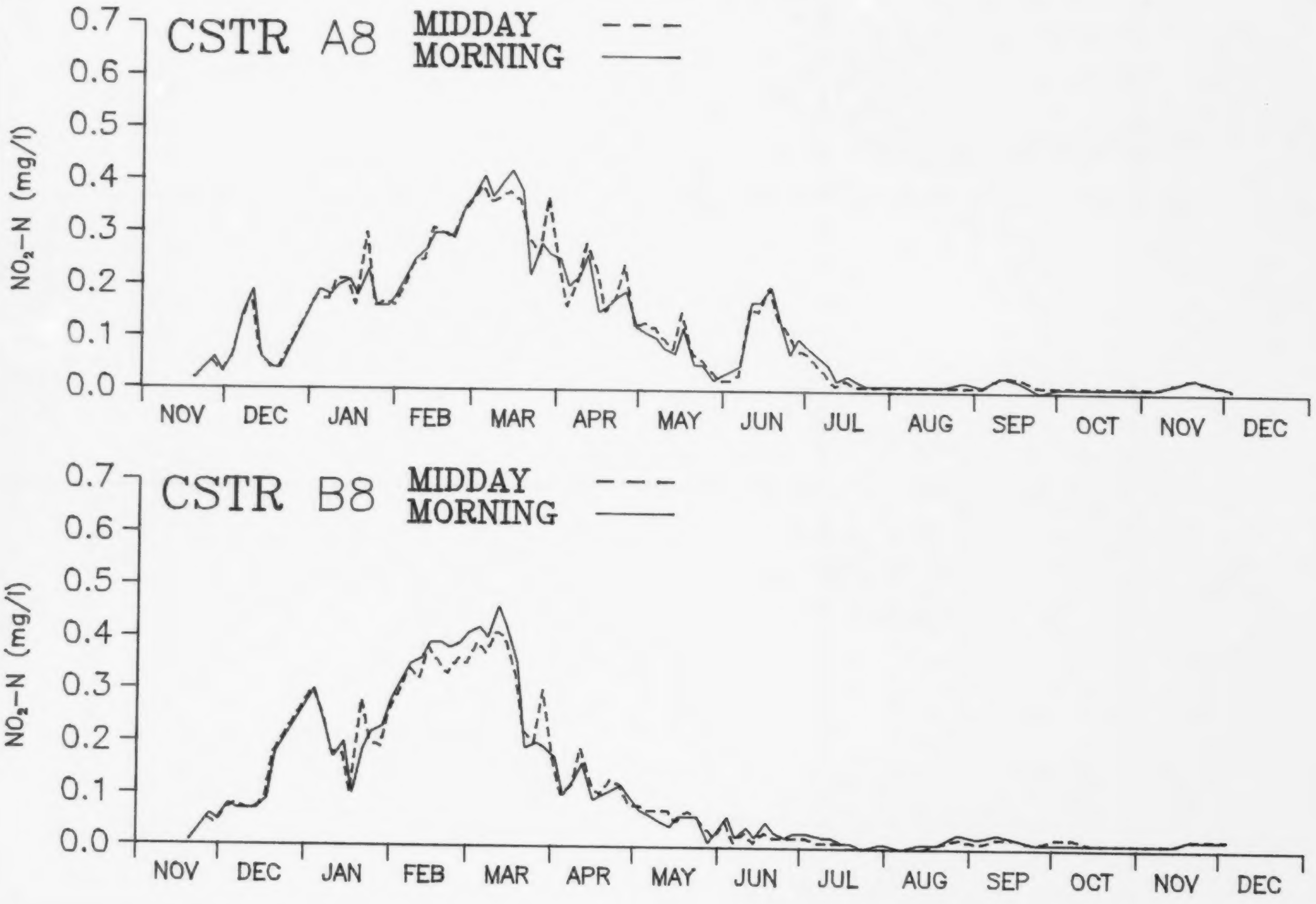


Figure 23. Nitrite-Nitrogen Concentrations in CSTRs A8 and B8

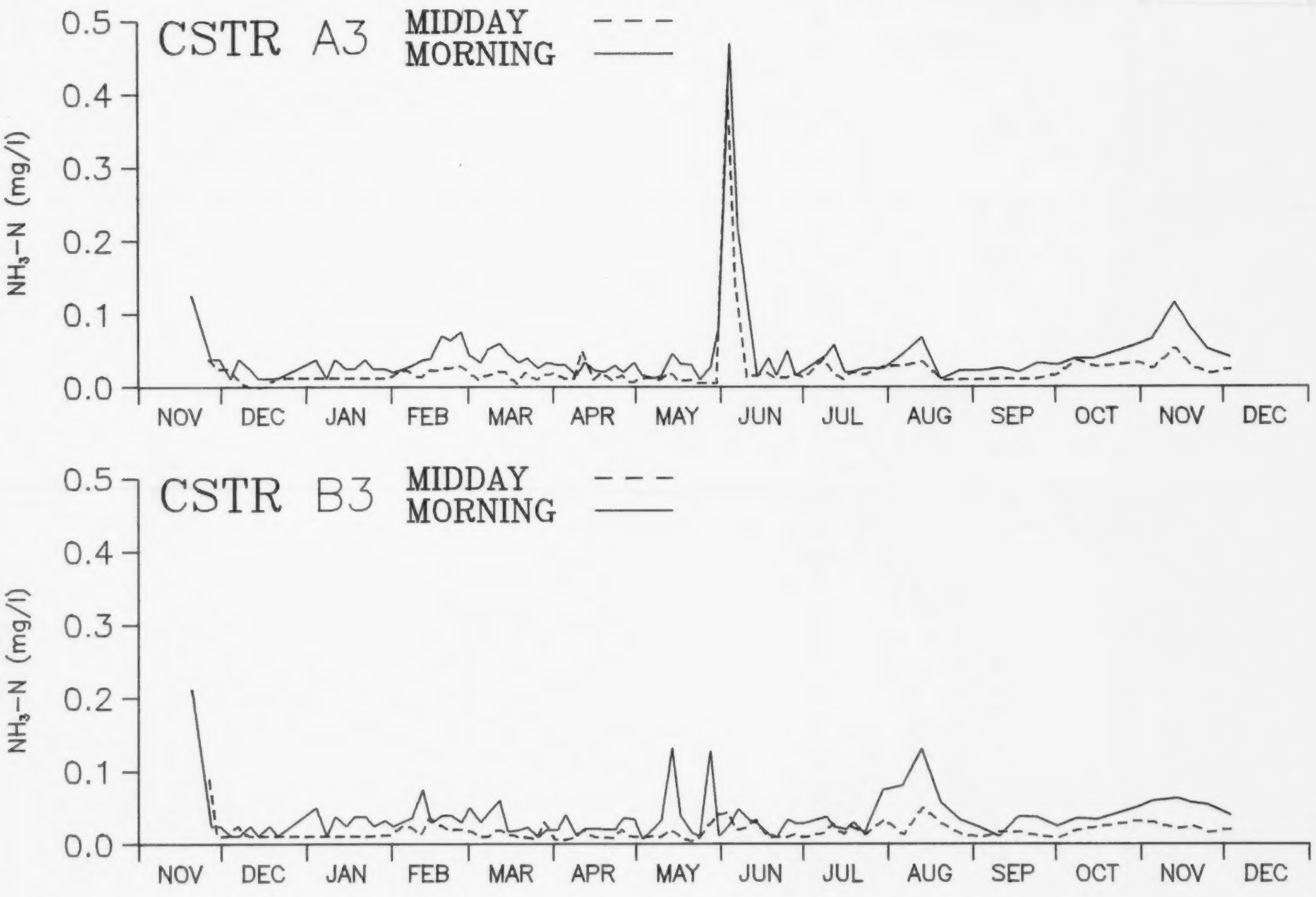


Figure 24. Ammonia-Nitrogen Concentrations in CSTRs A3 and B3

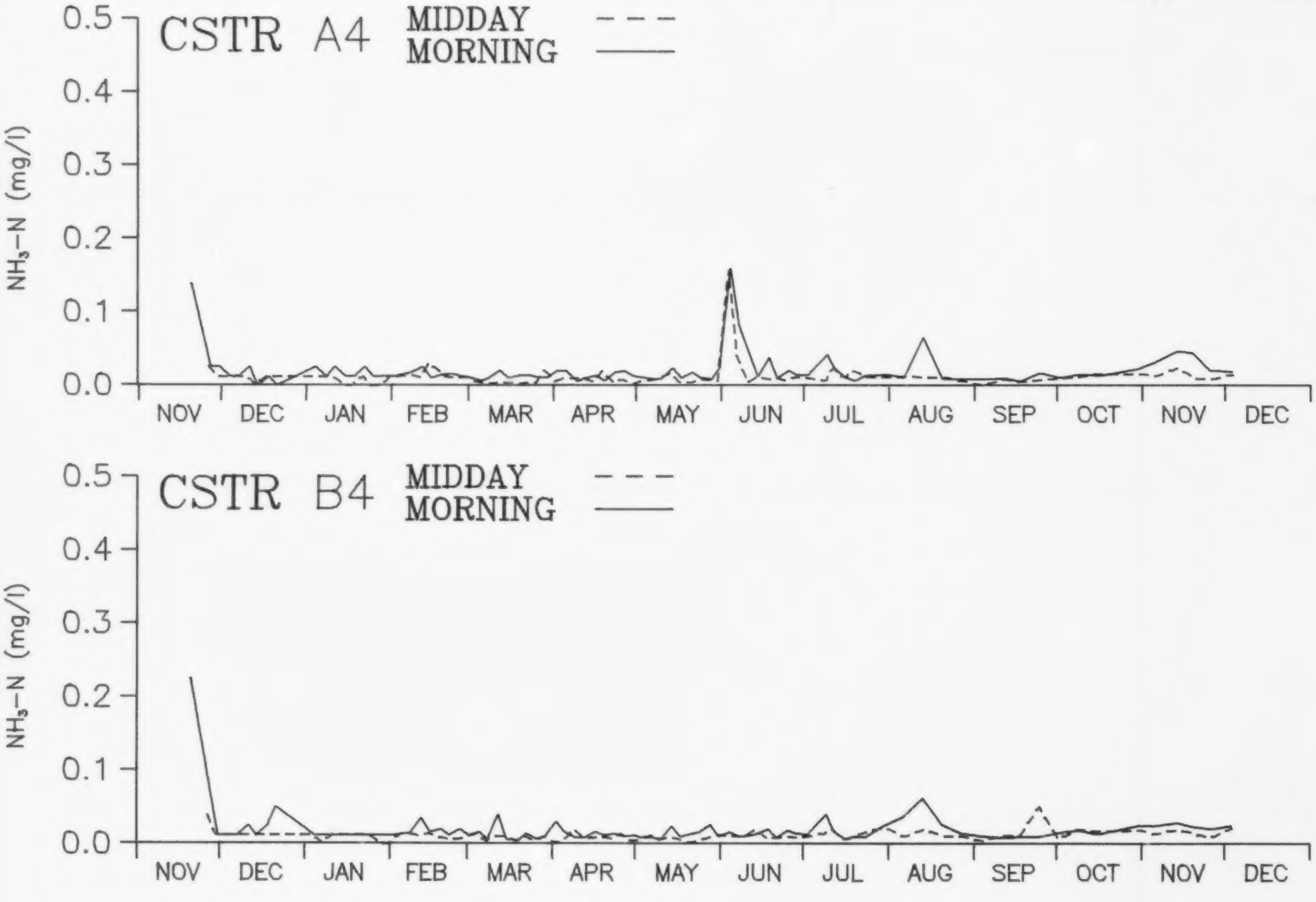


Figure 25. Ammonia-Nitrogen Concentrations in CSTRs A4 and B4

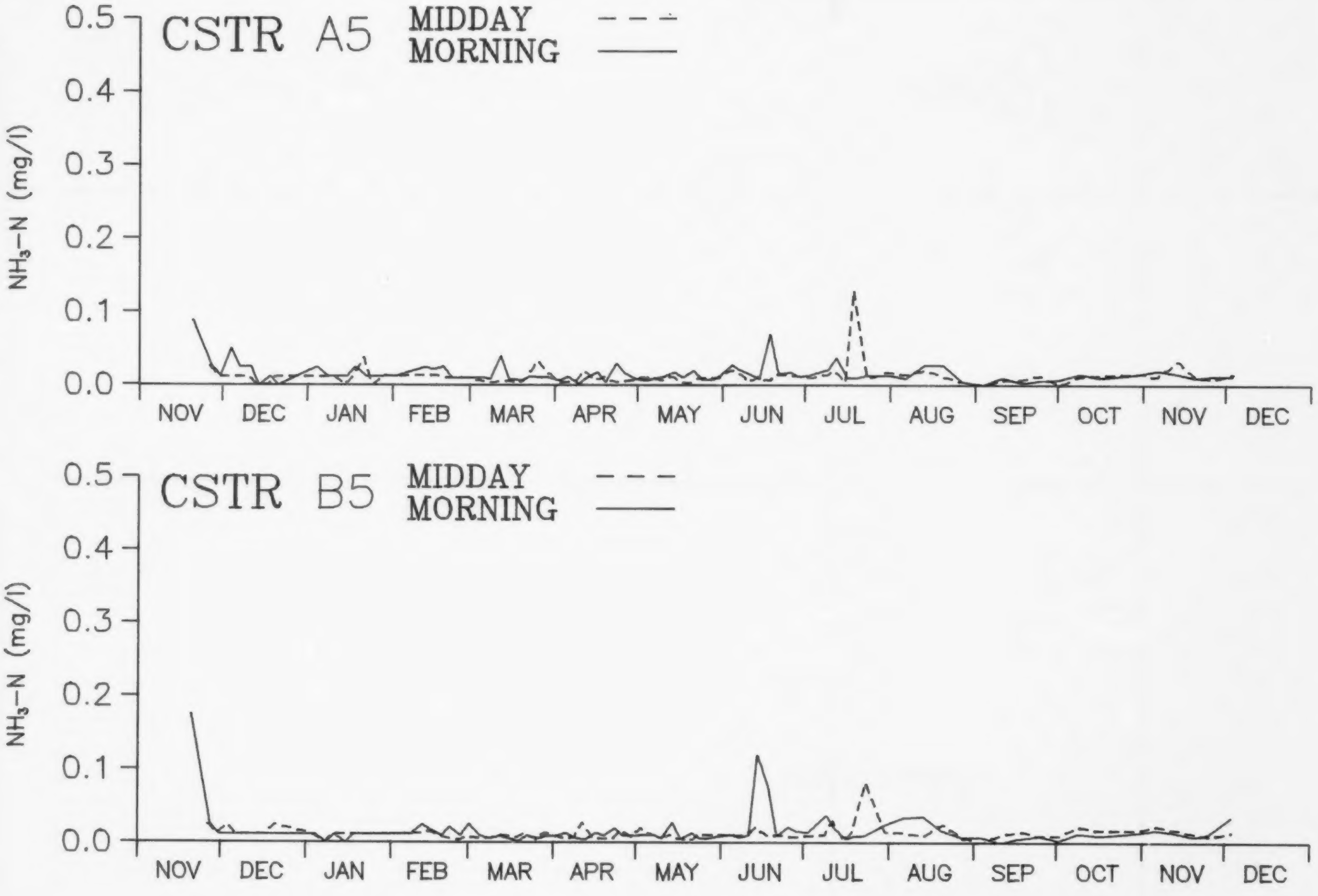


Figure 26. Ammonia-Nitrogen Concentrations in CSTRs A5 and B5

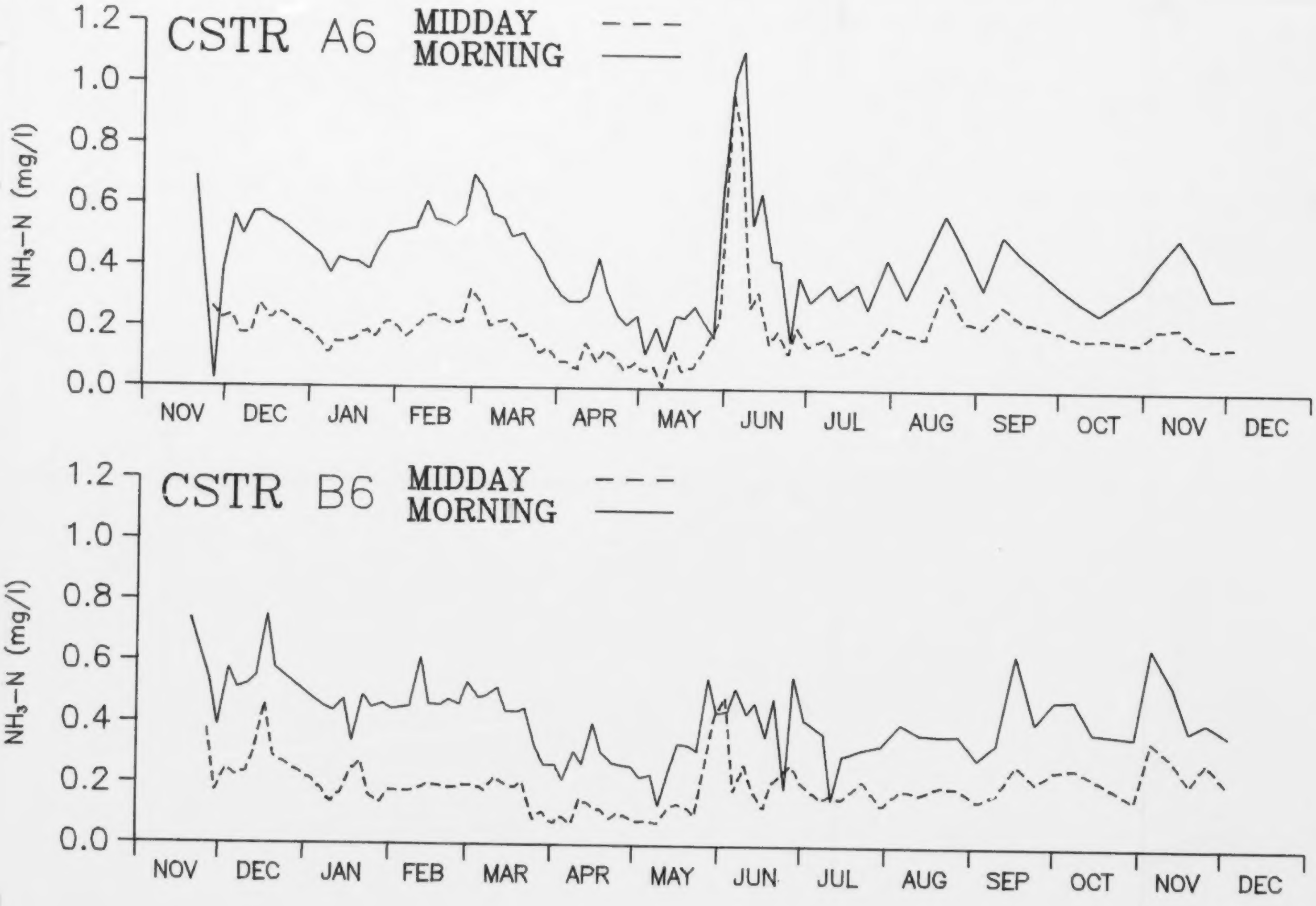


Figure 27. Ammonia-Nitrogen Concentrations in CSTRs A6 and B6

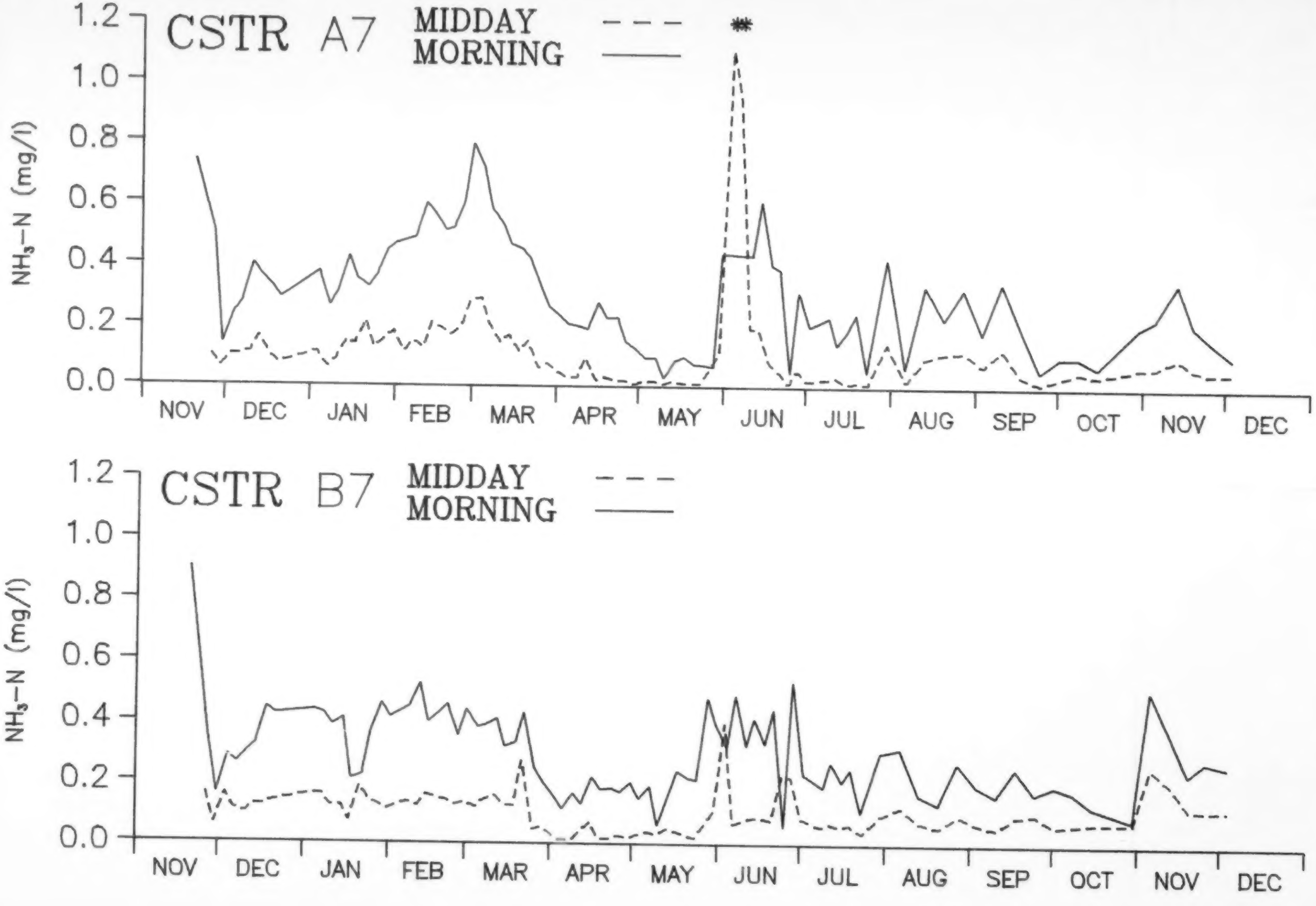


Figure 28. Ammonia-Nitrogen Concentrations in CSTRs A7 and B7

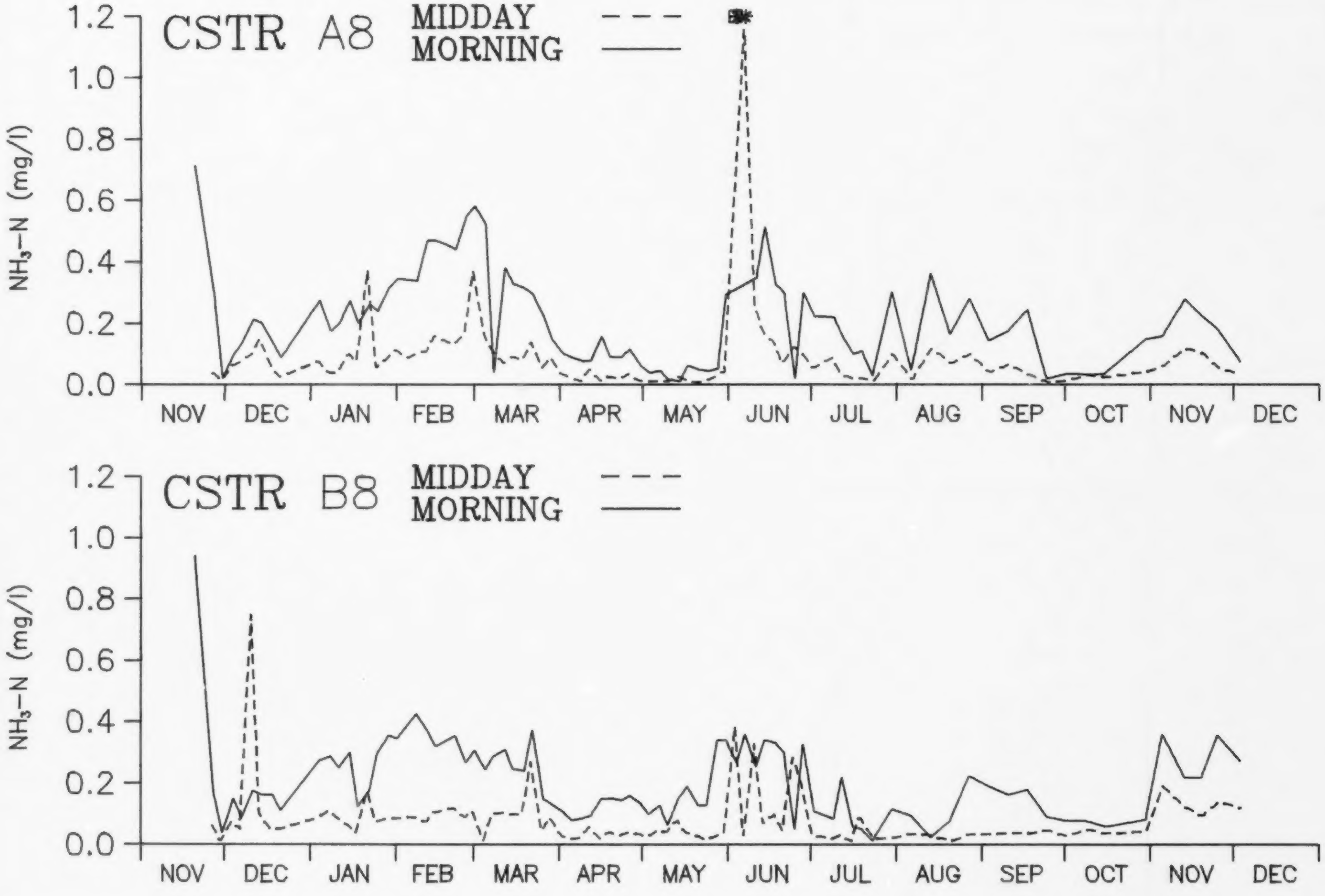


Figure 29. Ammonia-Nitrogen Concentrations in CSTRs A8 and B8

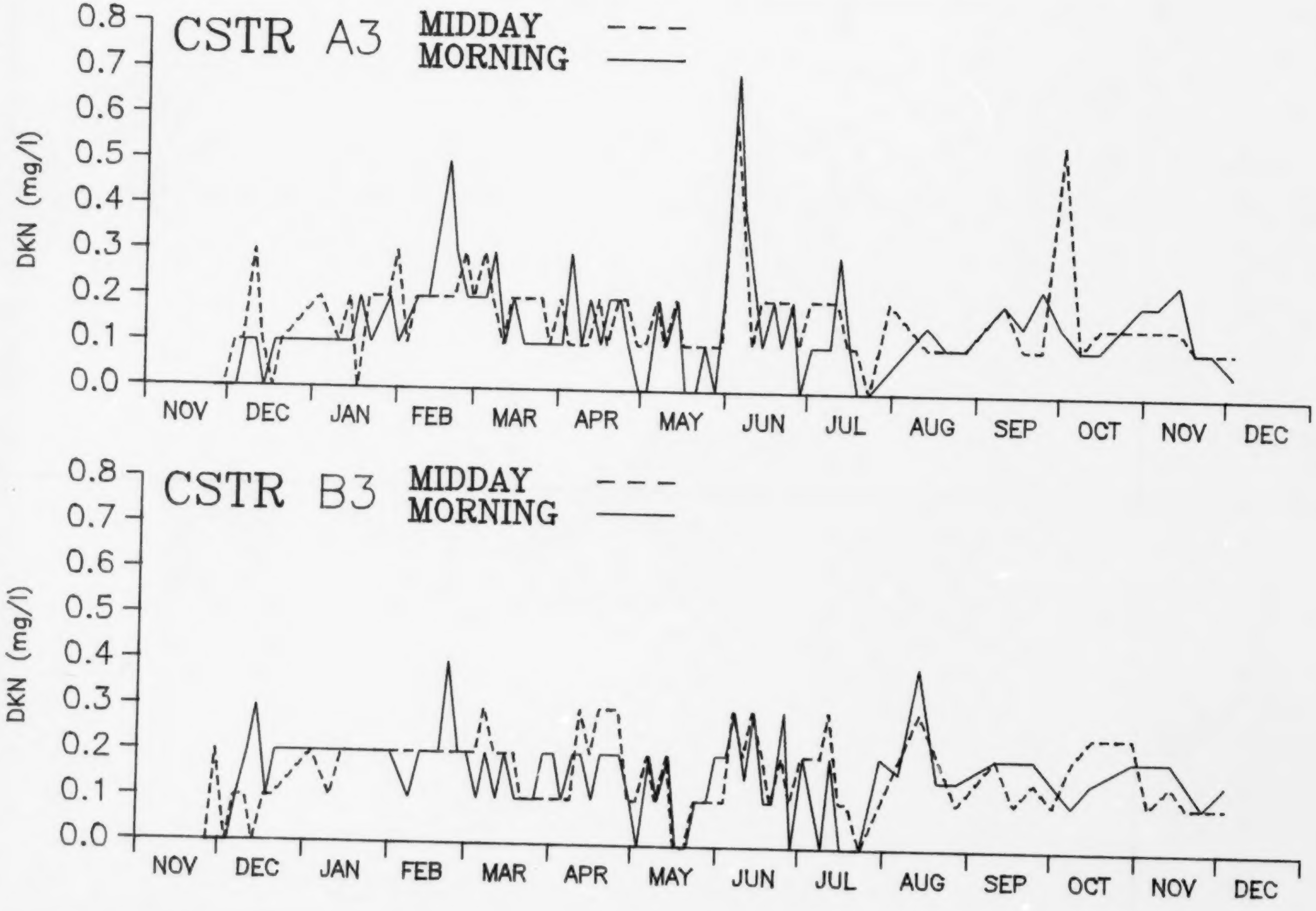


Figure 30. Dissolved Kjeldahl Nitrogen Concentrations in CSTRs A3 and B3

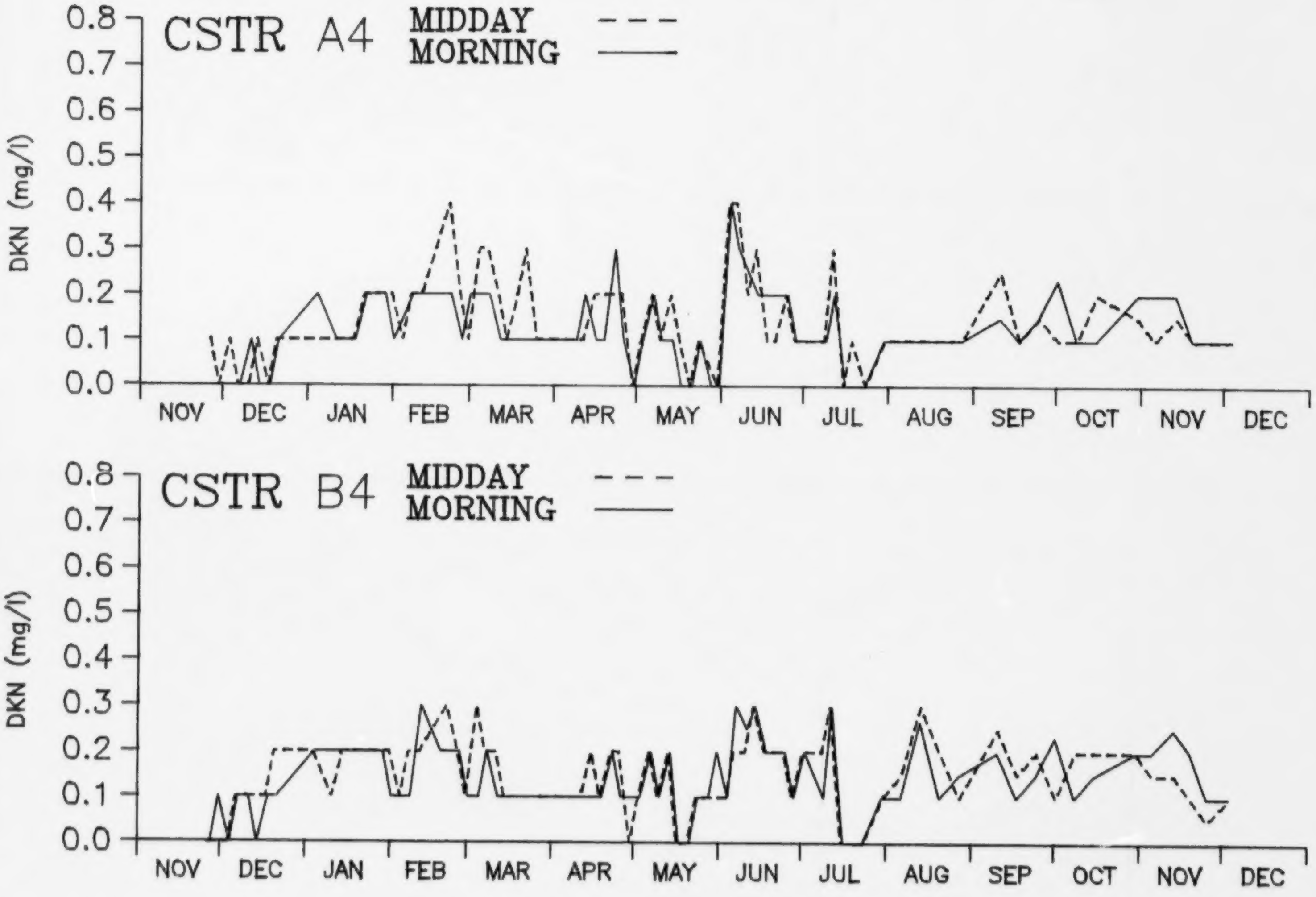


Figure 31. Dissolved Kjeldahl Nitrogen Concentrations in CSTRs A4 and B4

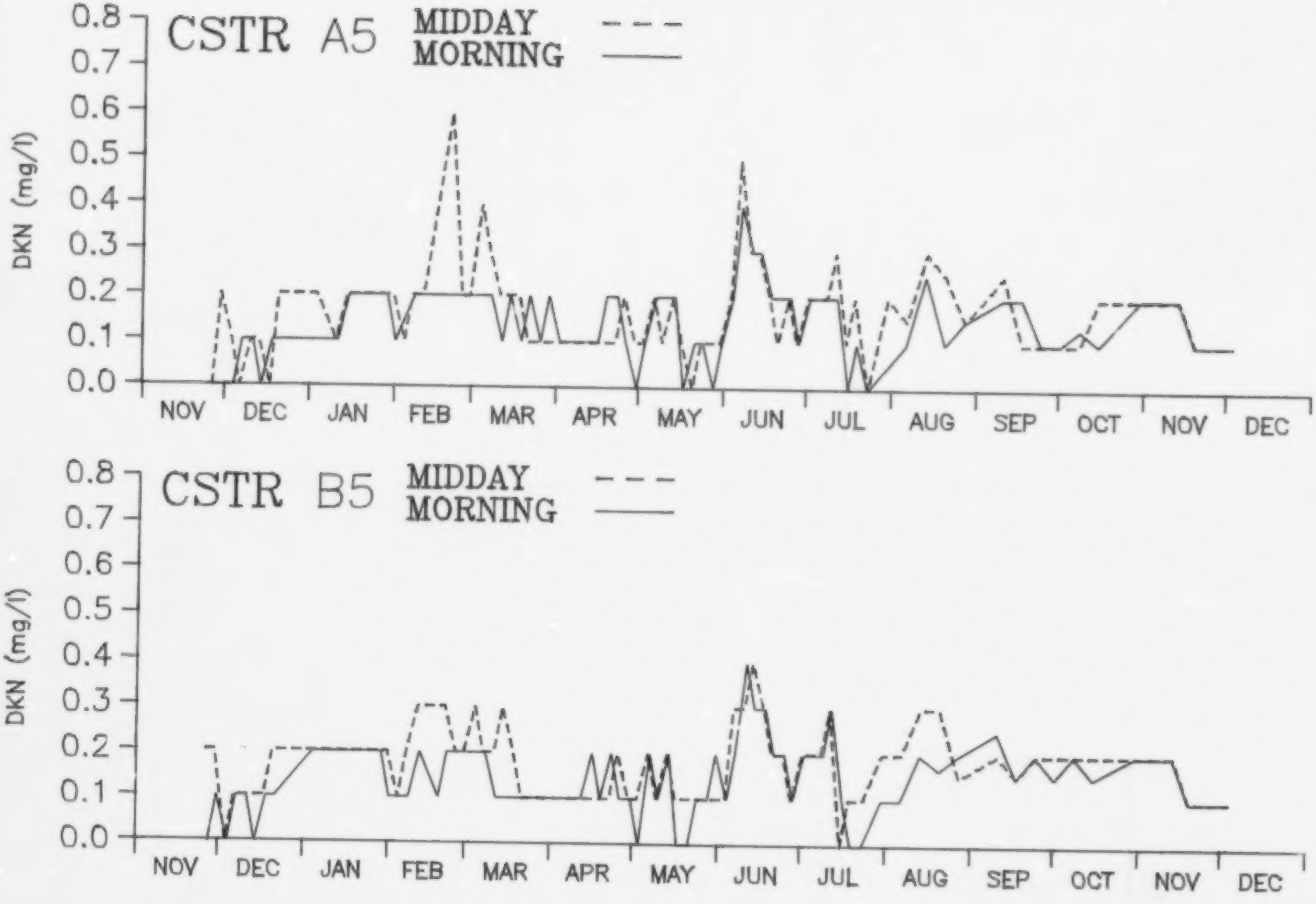


Figure 32. Dissolved Kjeldahl Nitrogen Concentrations in CSTRs A5 and B5

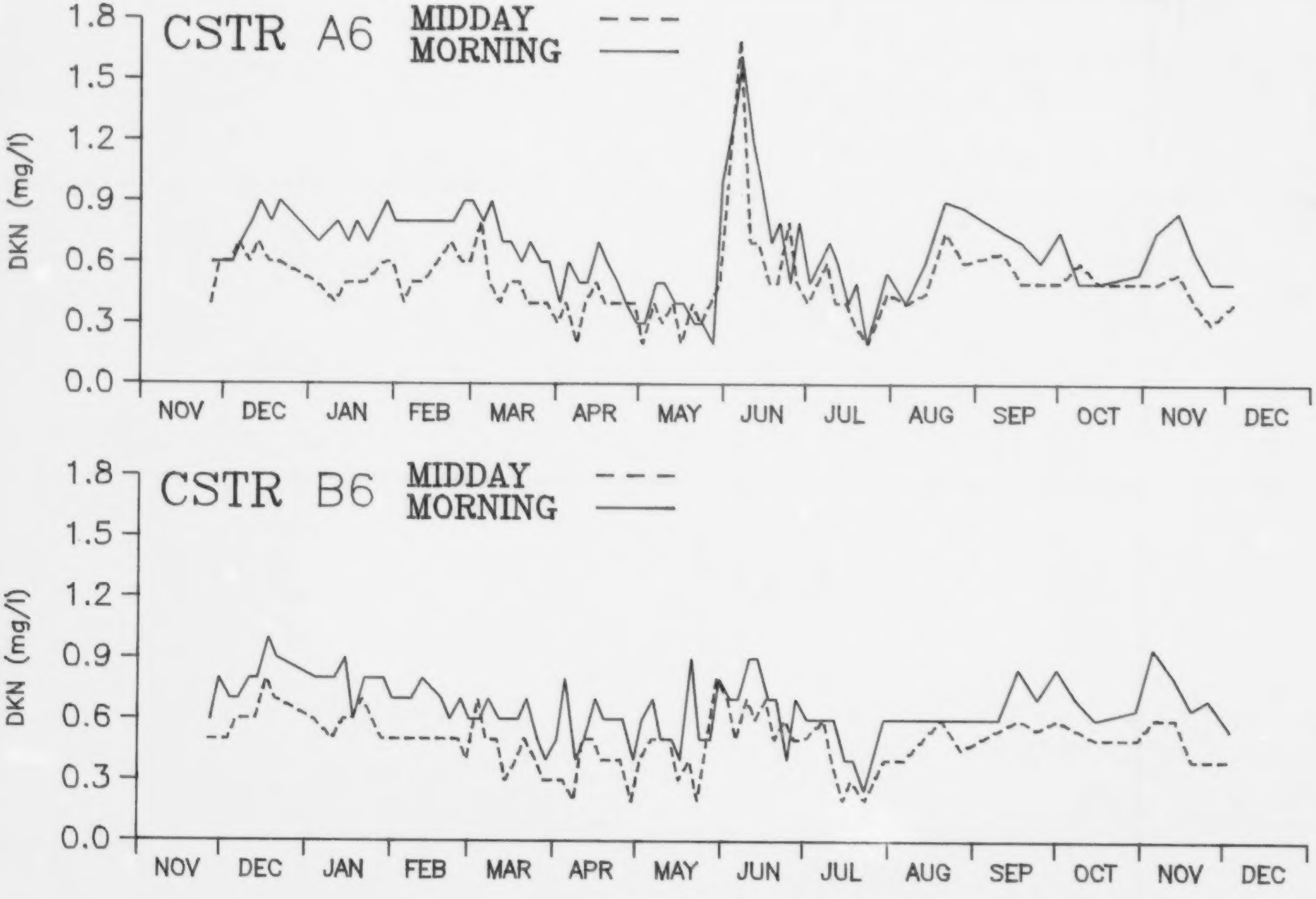


Figure 33. Dissolved Kjeldahl Nitrogen Concentrations in CSTRs A6 and B6

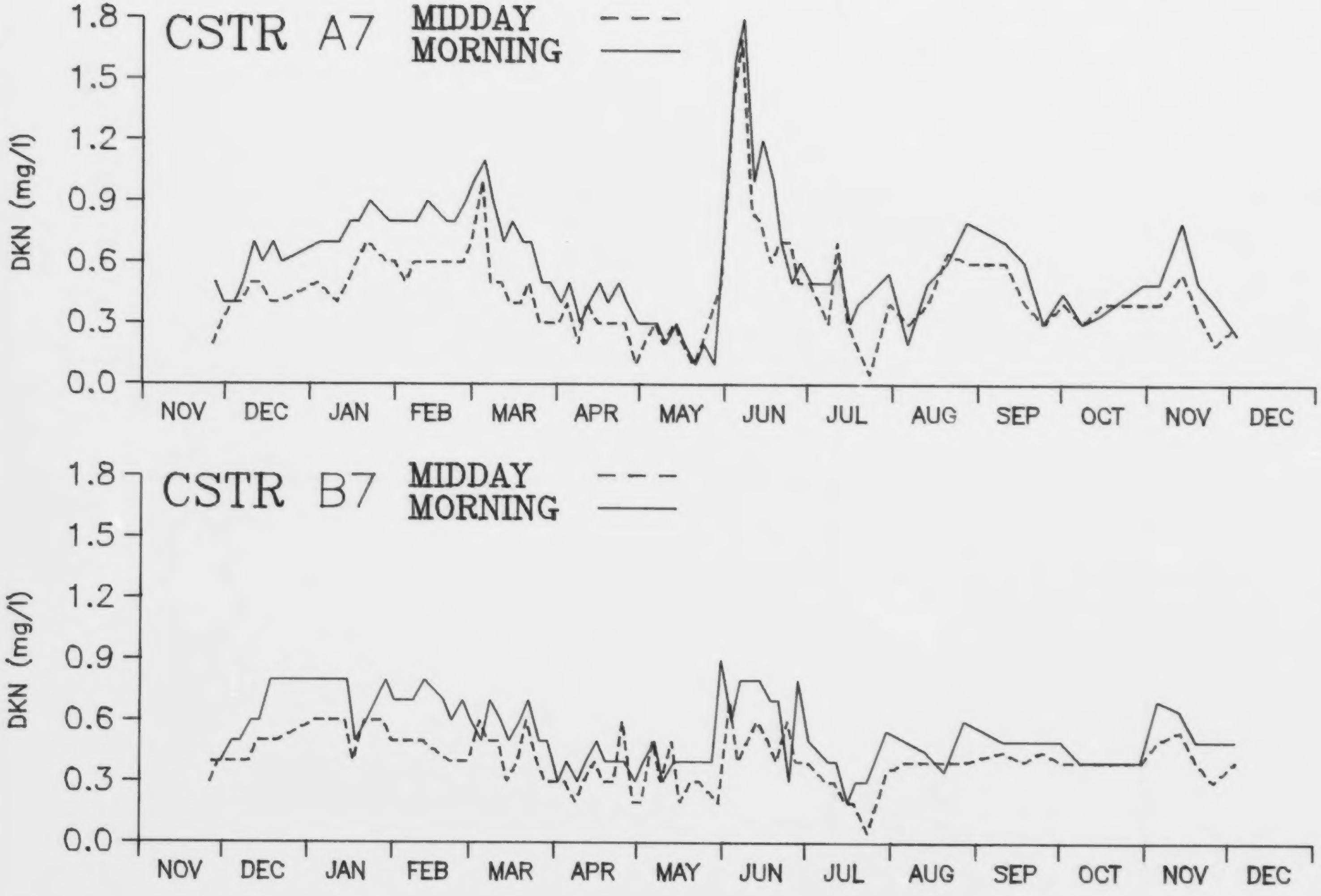


Figure 34. Dissolved Kjeldahl Nitrogen Concentrations in CSTRs A7 and B7

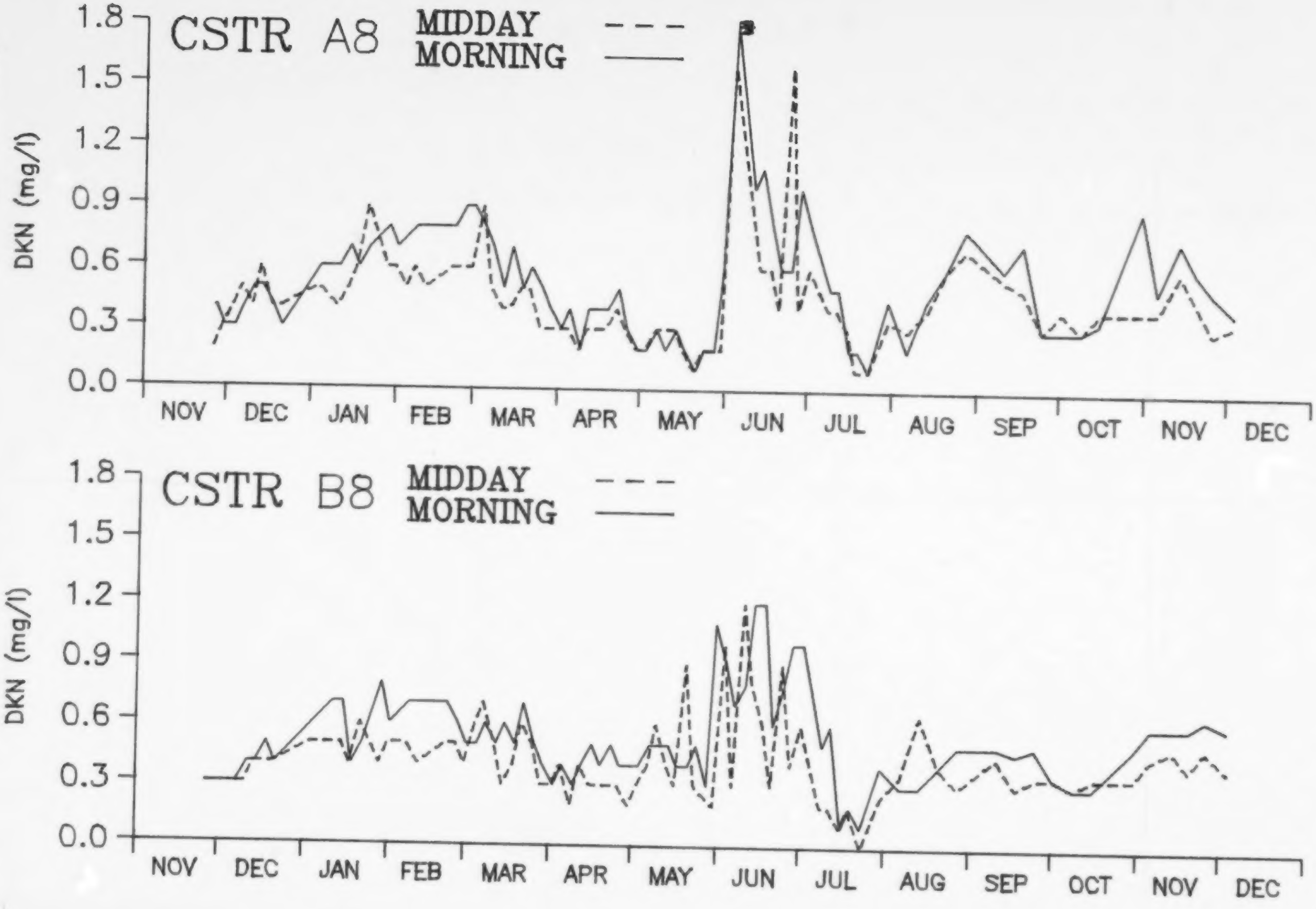


Figure 35. Dissolved Kjeldahl Nitrogen Concentrations in CSTRs A8 and B8

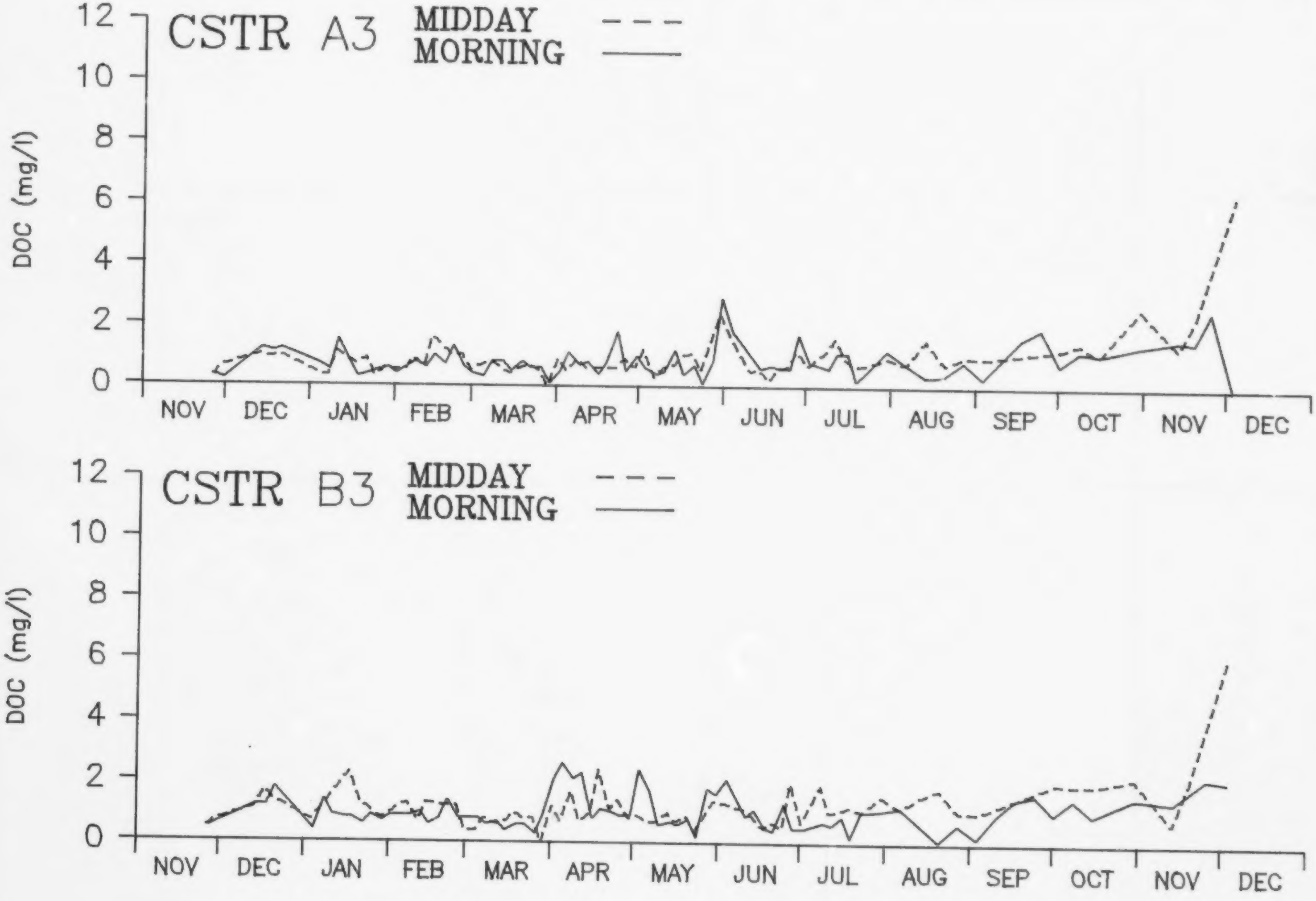


Figure 36. Dissolved Organic Carbon Concentrations in CSTRs A3 and B3

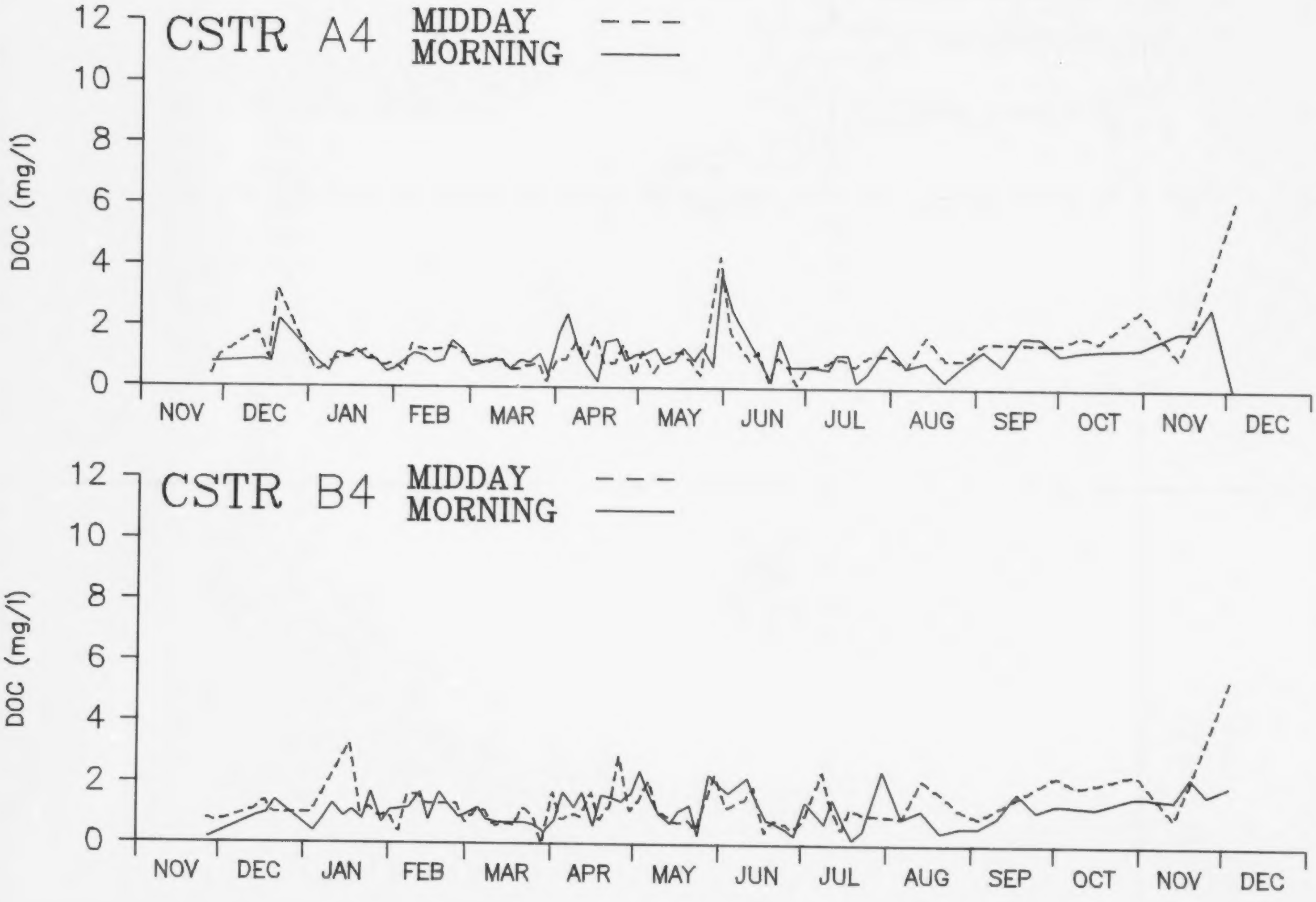


Figure 37. Dissolved Organic Carbon Concentrations in CSTRs A4 and B4

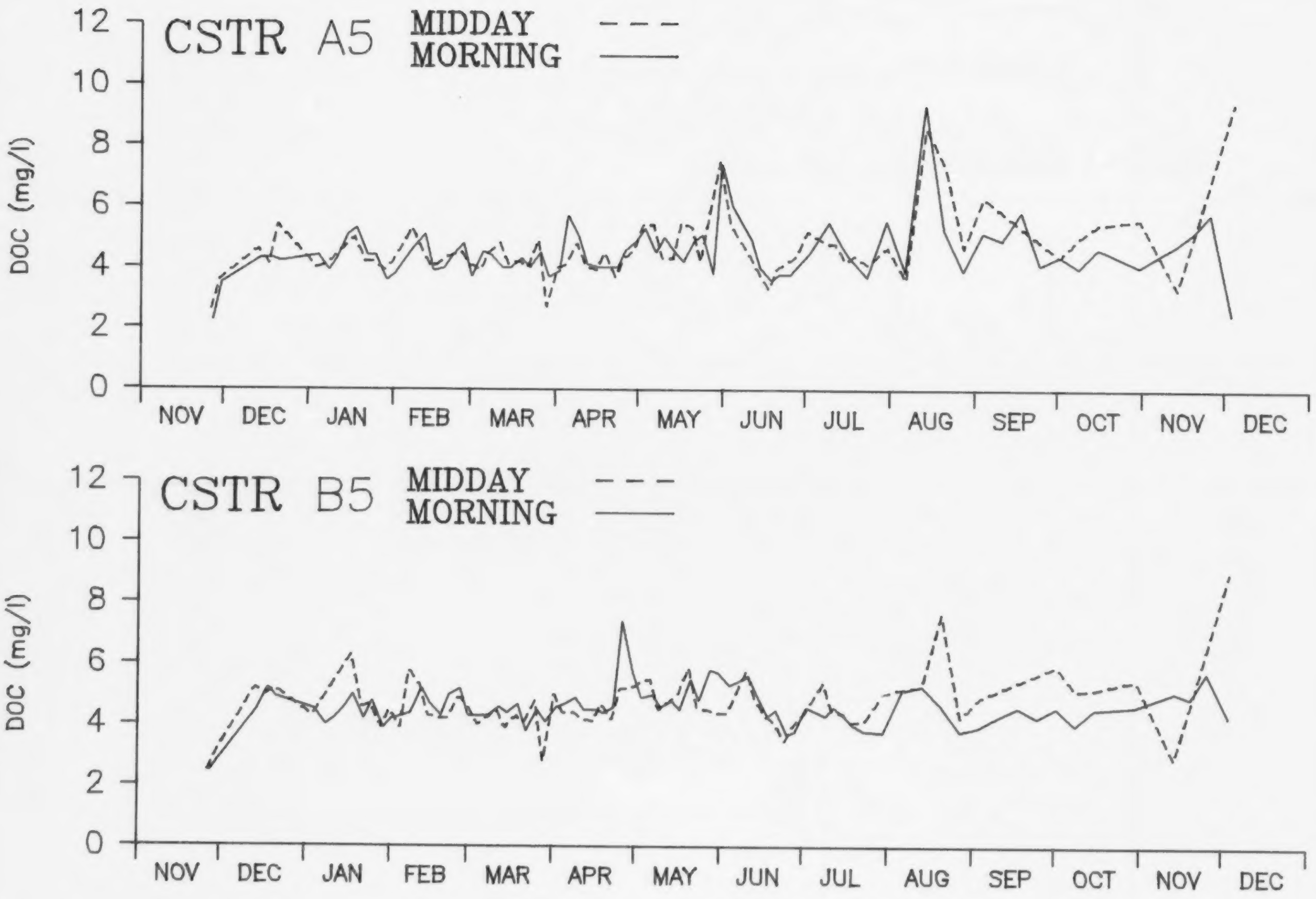


Figure 38. Dissolved Organic Carbon Concentrations in CSTRs A5 and B5

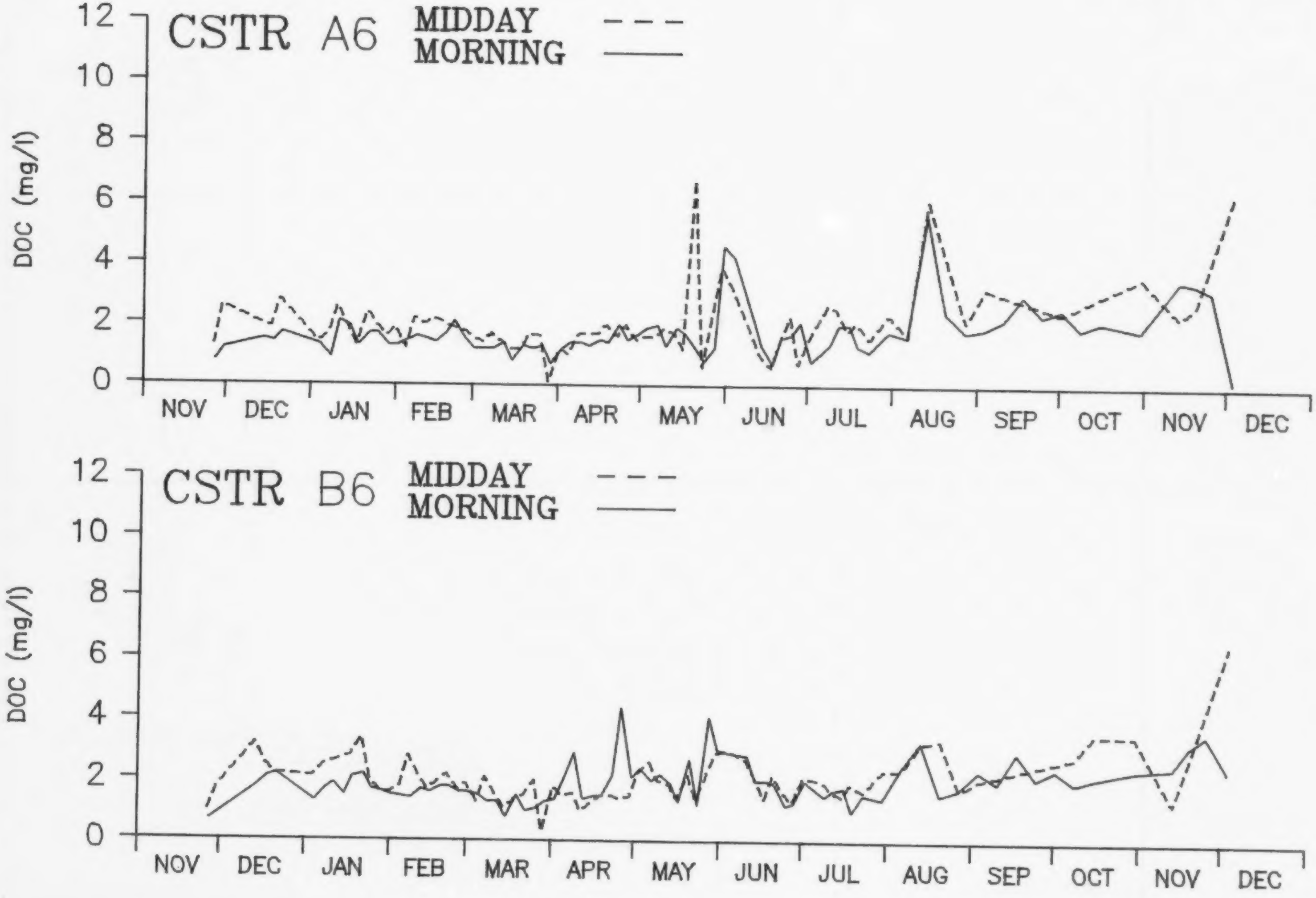


Figure 39. Dissolved Organic Carbon Concentrations in CSTRs A6 and B6

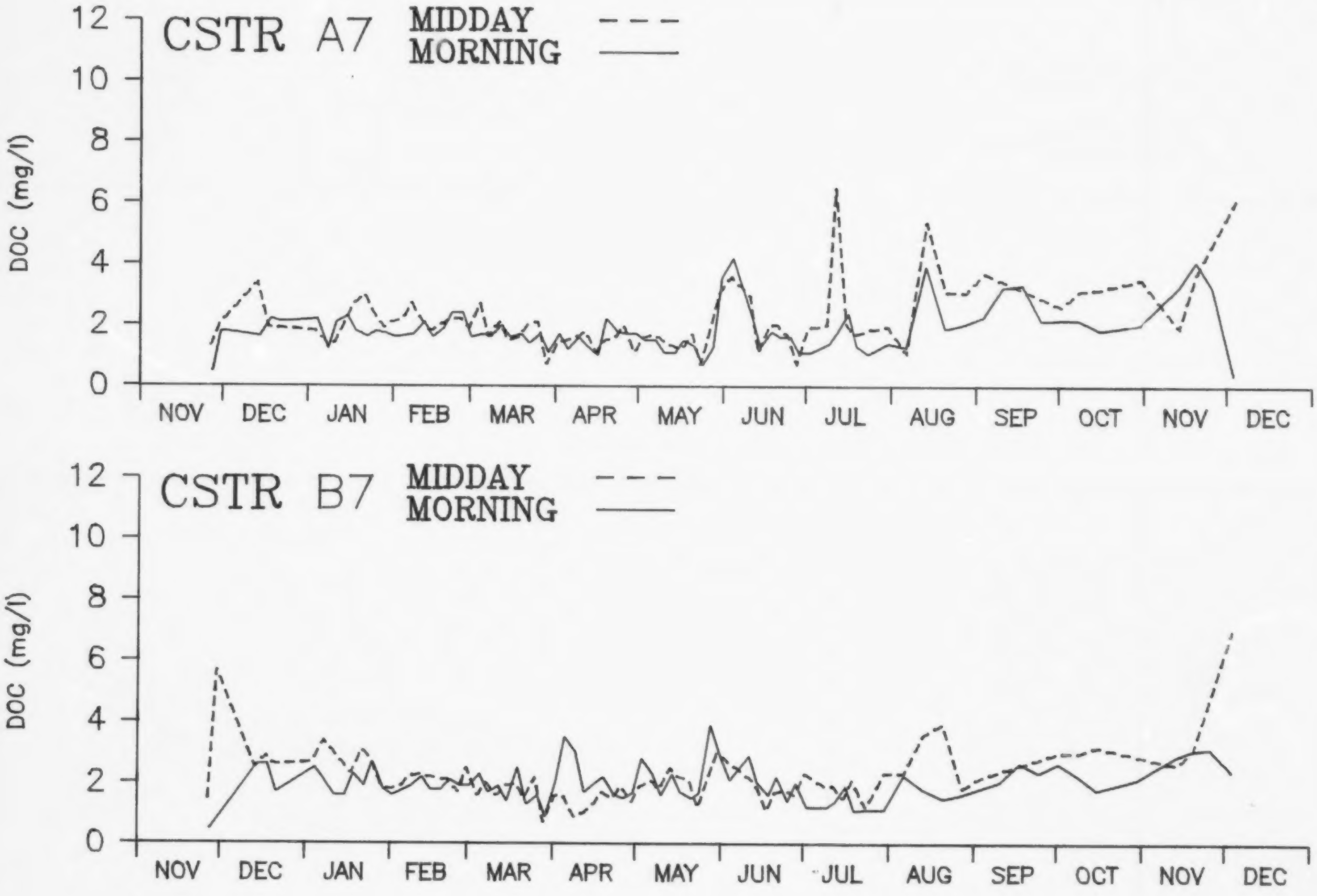


Figure 40. Dissolved Organic Carbon Concentrations in CSTRs A7 and B7

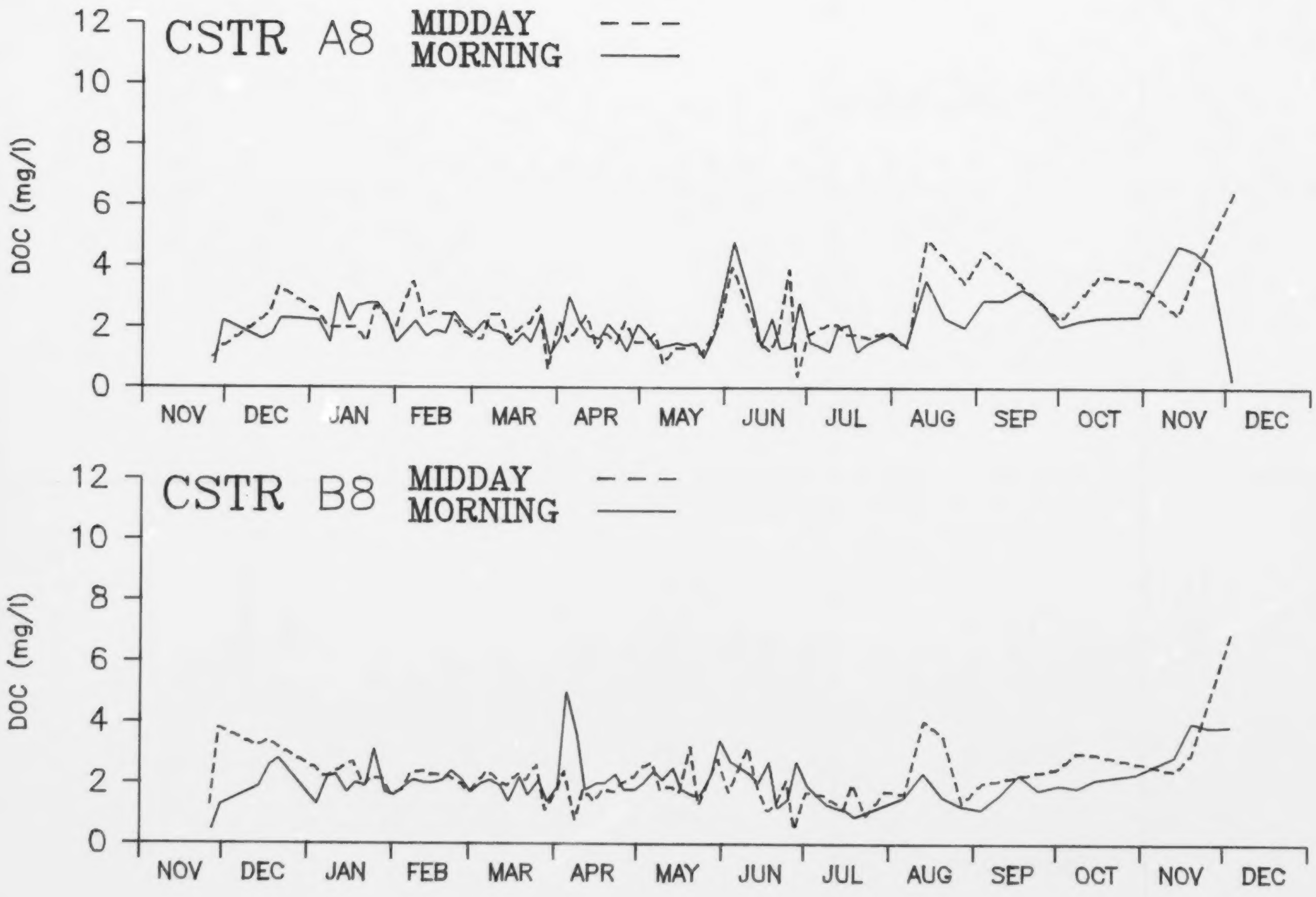


Figure 41. Dissolved Organic Carbon Concentrations in CSTRs A8 and B8

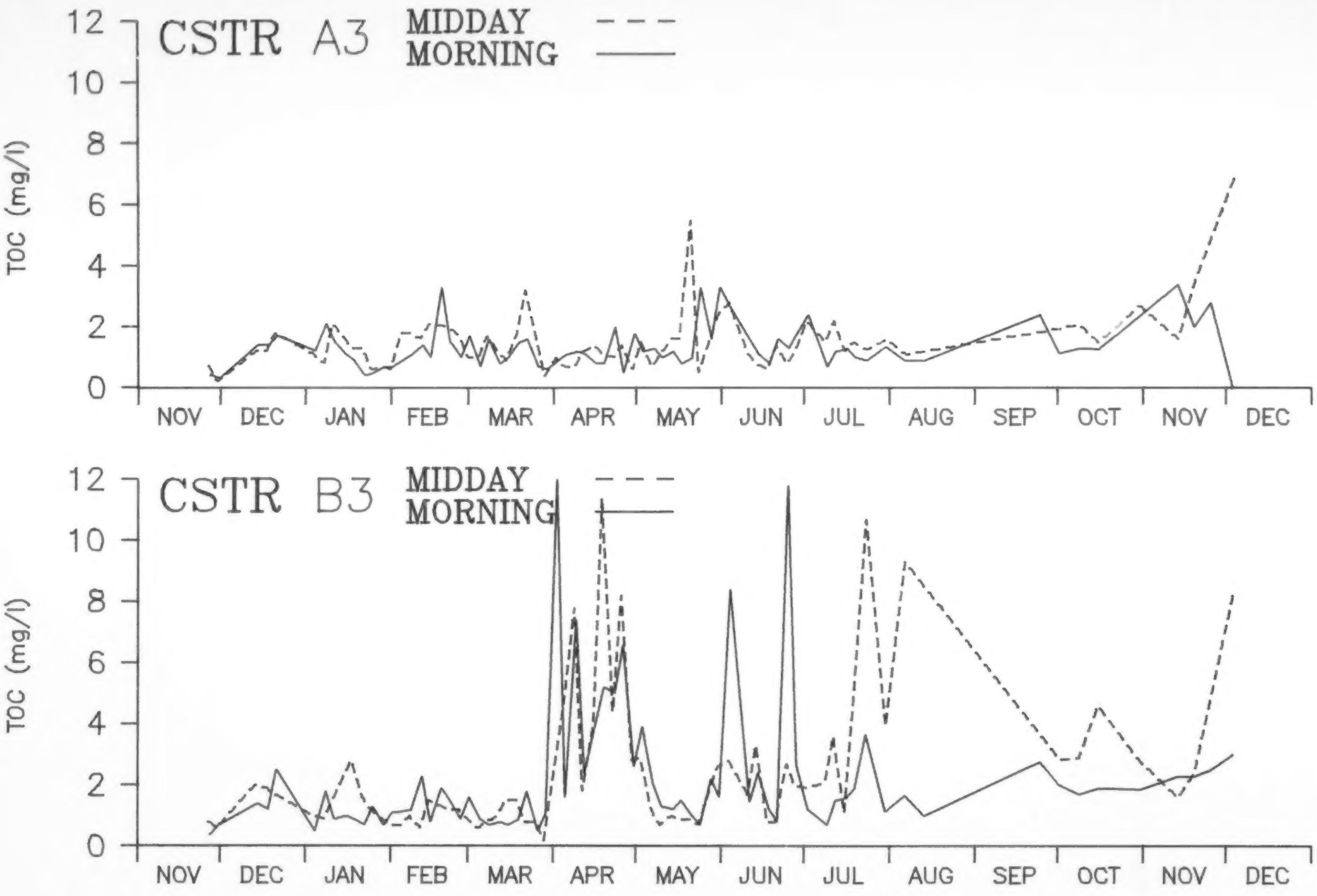


Figure 42. Total Organic Carbon Concentrations in CSTRs A3 and B3

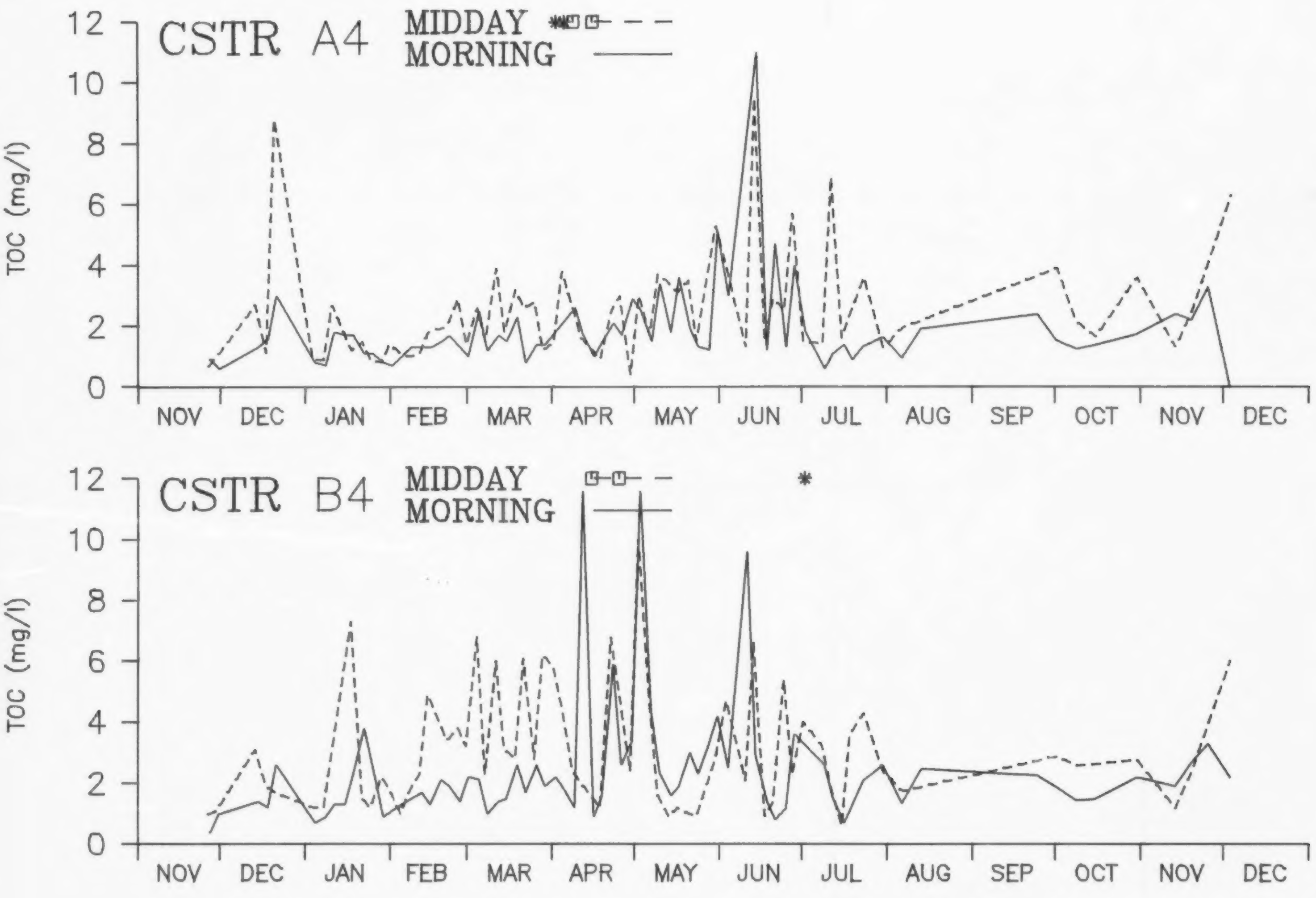


Figure 43. Total Organic Carbon Concentrations in CSTRs A4 and B4

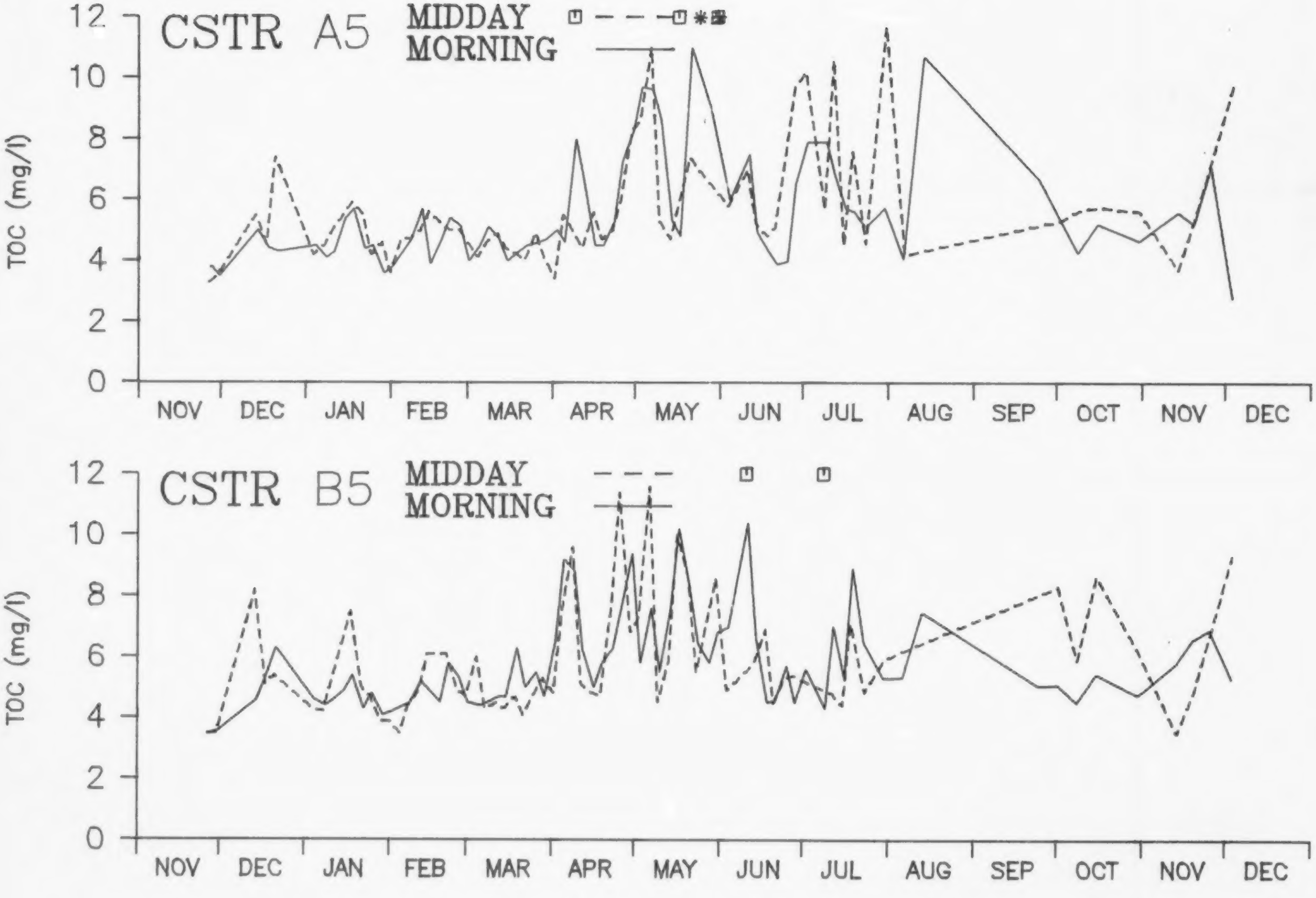


Figure 44. Total Organic Carbon Concentrations in CSTRs A5 and B5

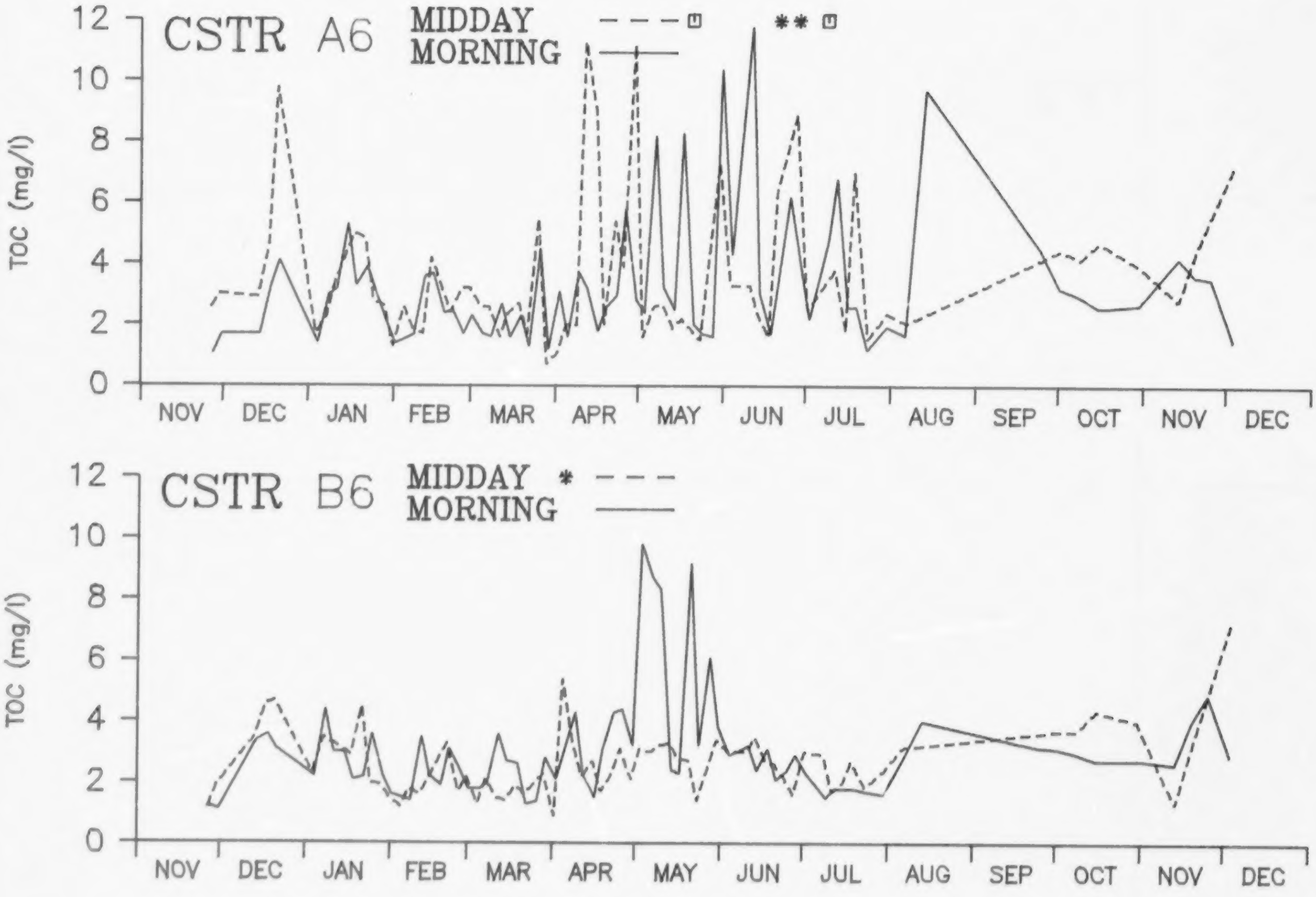


Figure 45. Total Organic Carbon Concentrations in CSTRs A6 and B6

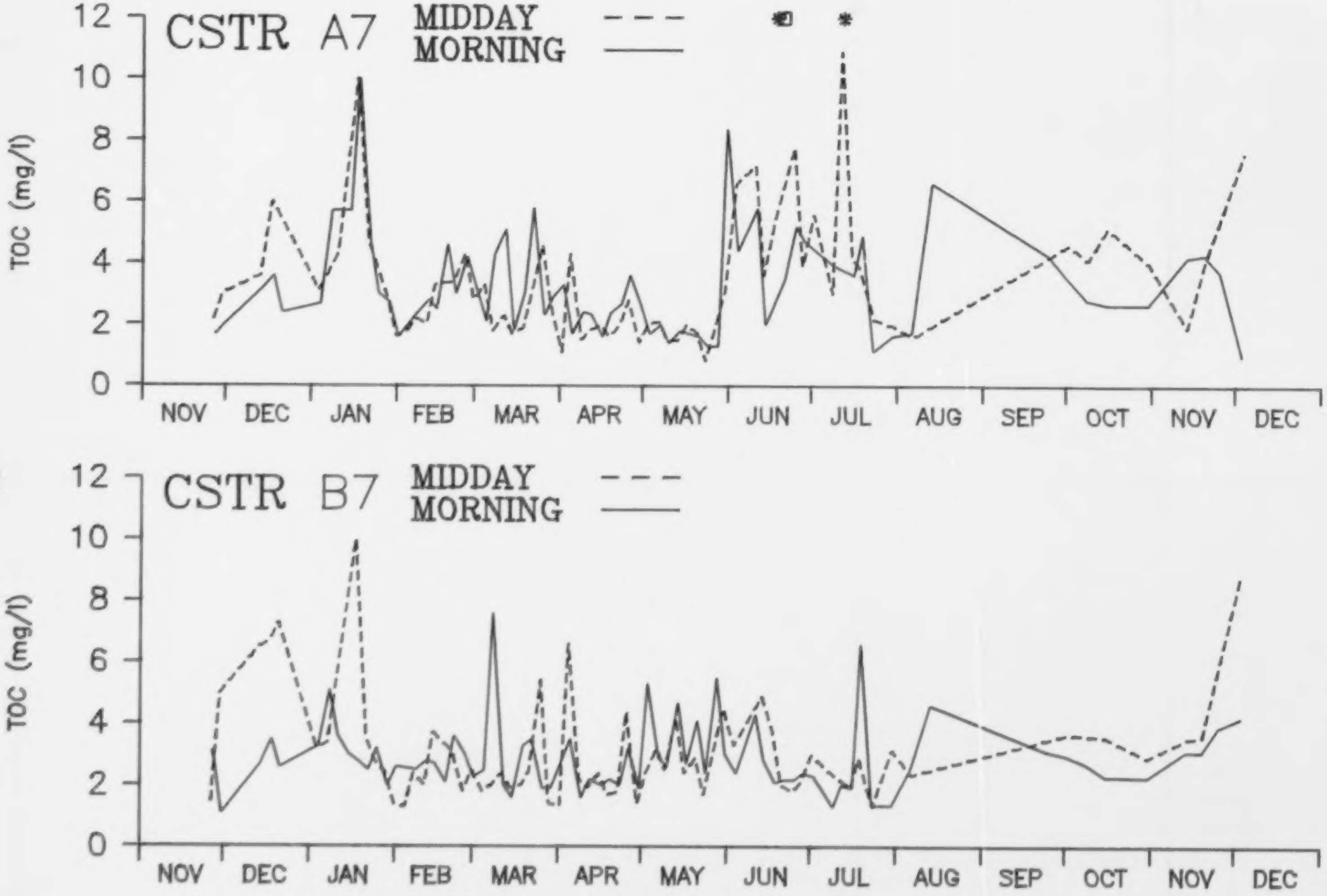


Figure 46. Total Organic Carbon Concentrations in CSTRs A7 and B7

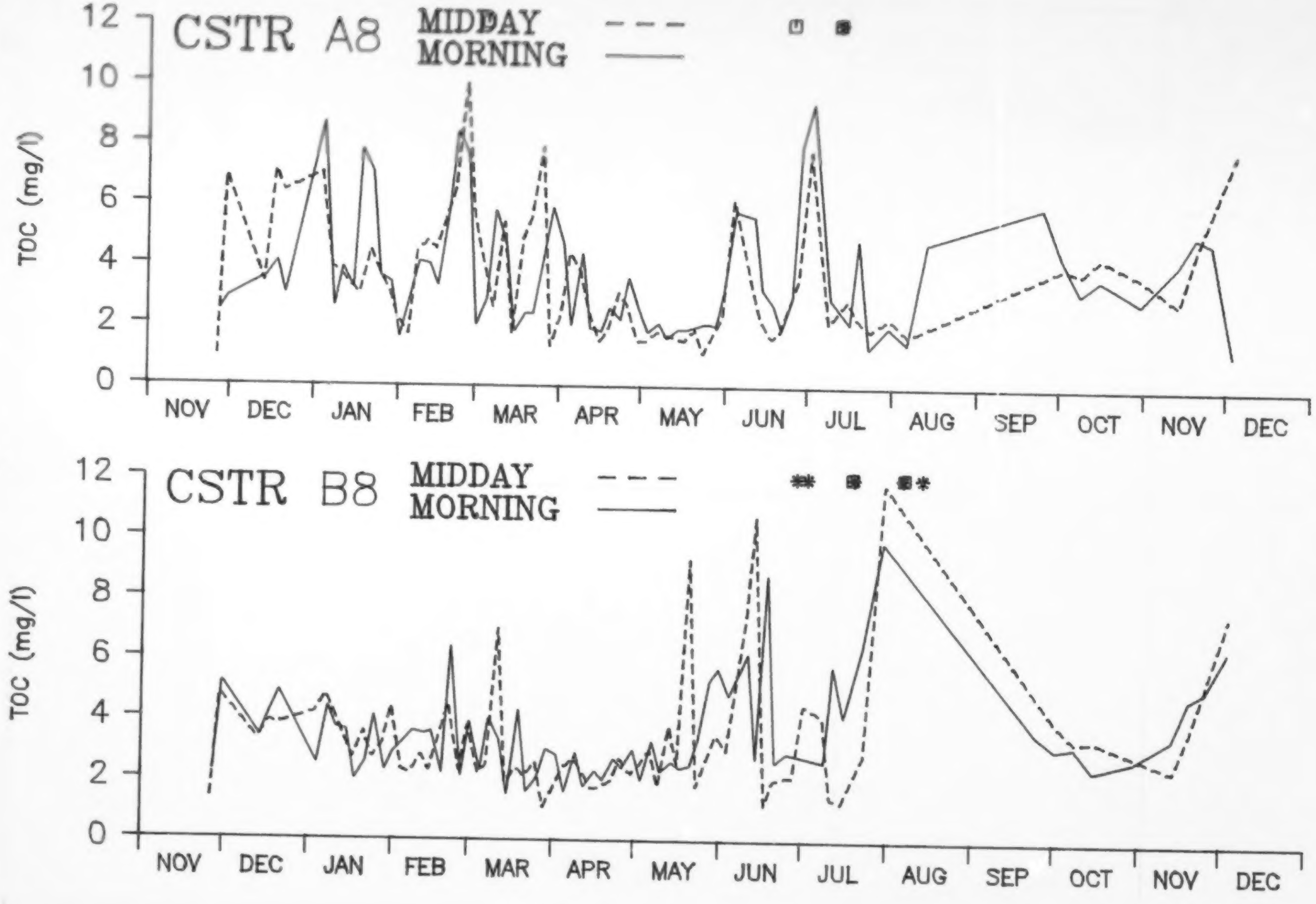


Figure 47. Total Organic Carbon Concentrations in CSTRs A8 and B8

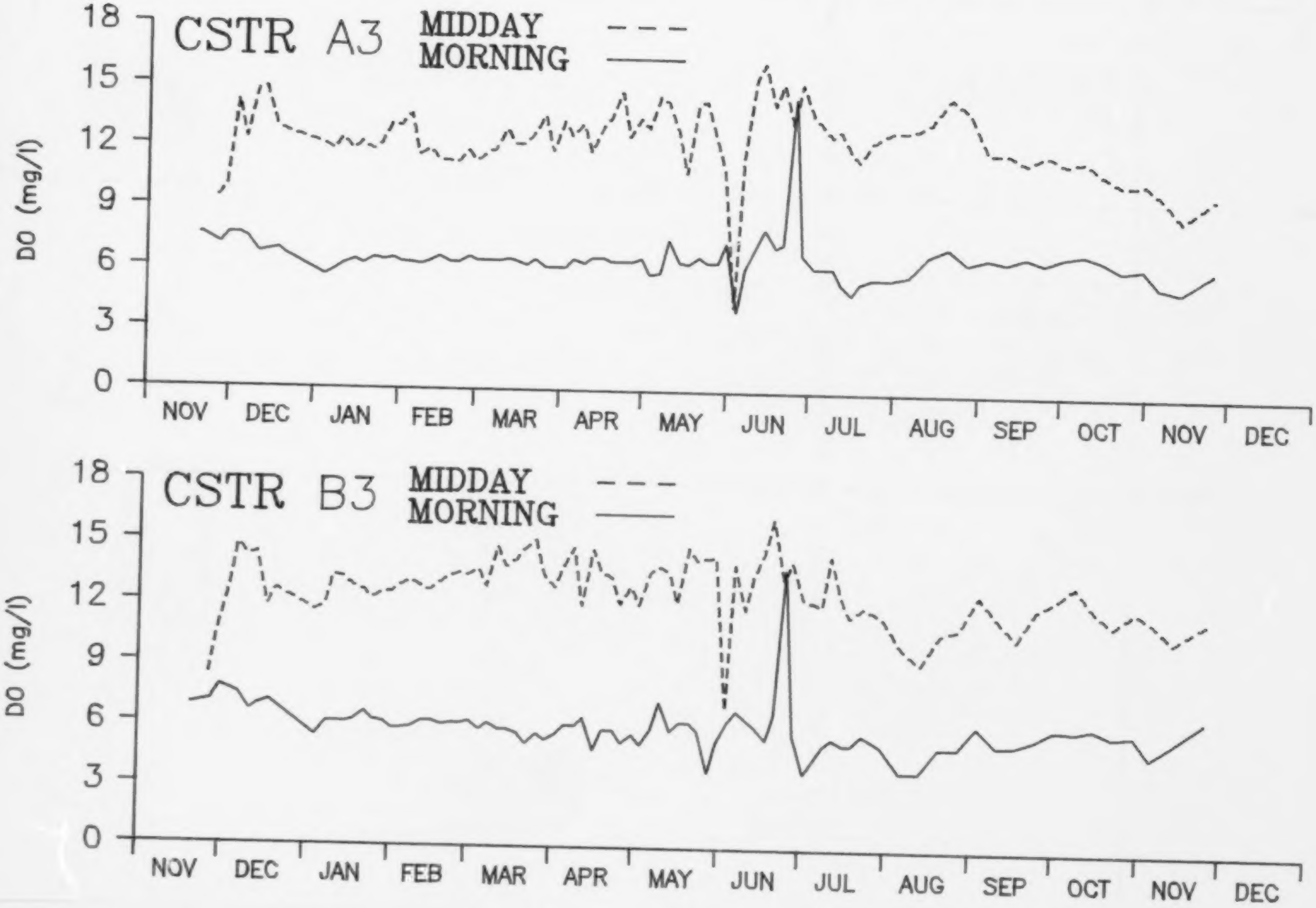


Figure 48. Dissolved Oxygen Concentrations in A3 and B3

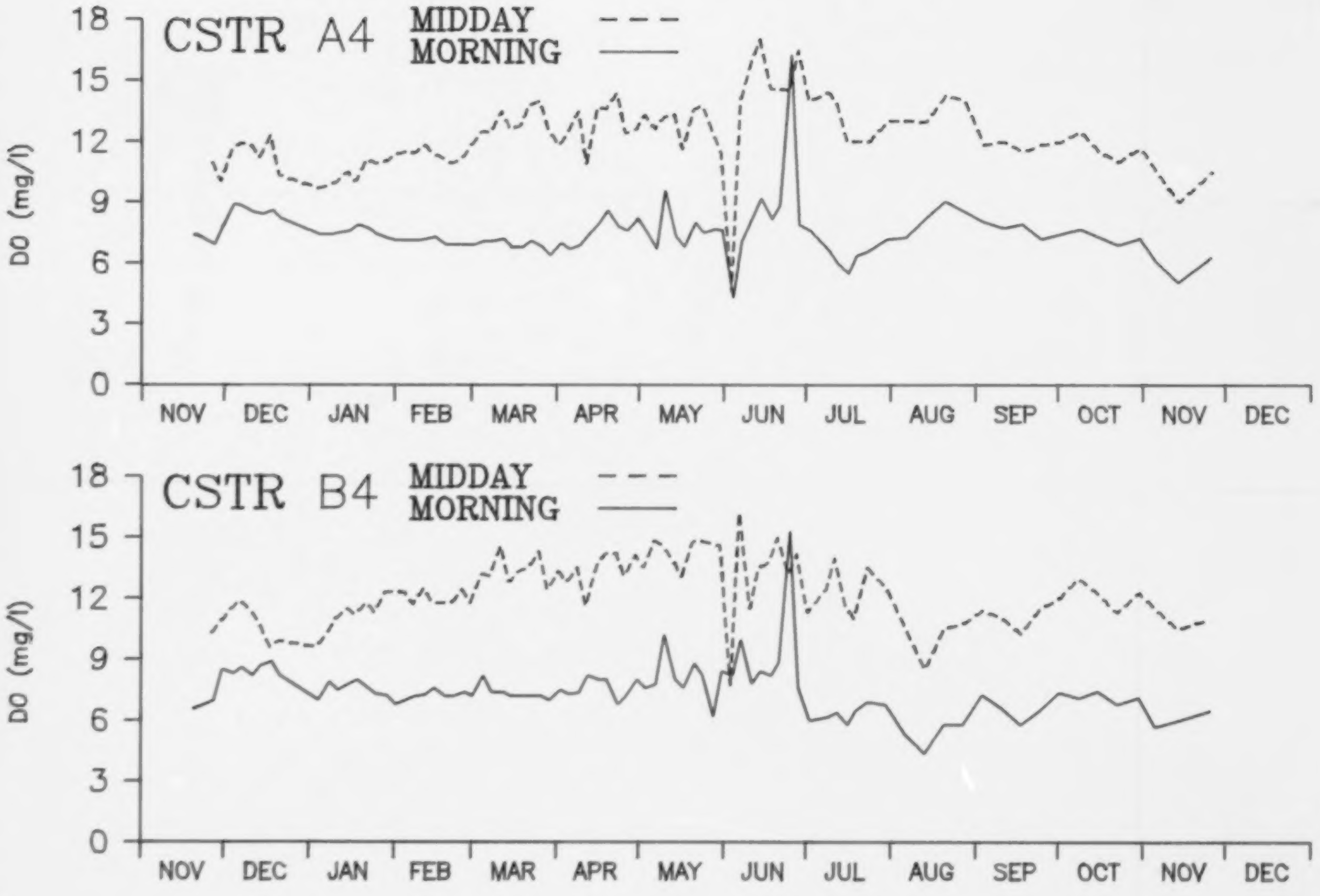


Figure 49. Dissolved Oxygen Concentrations in A4 and B4

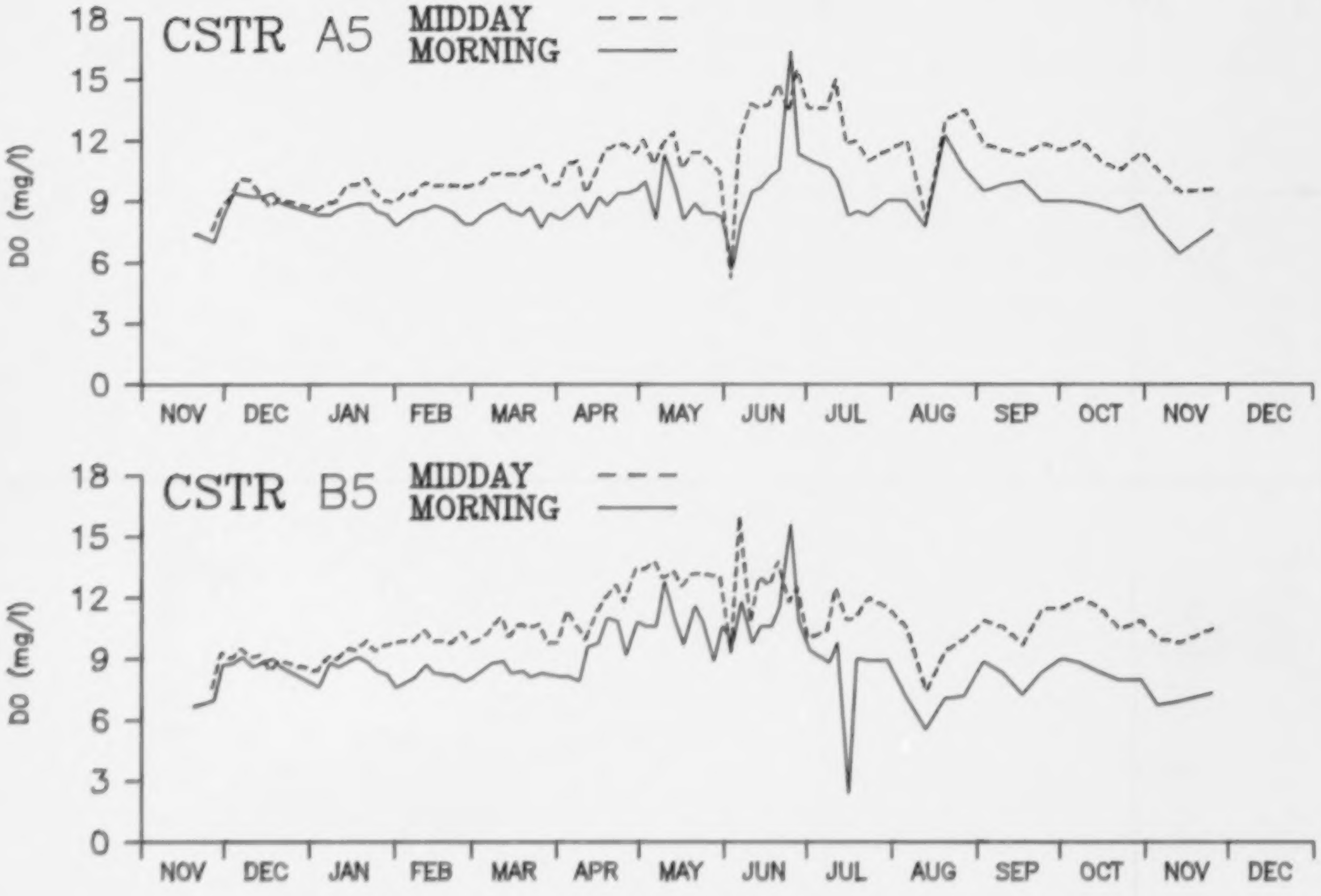


Figure 50. Dissolved Oxygen Concentrations in A5 and B5

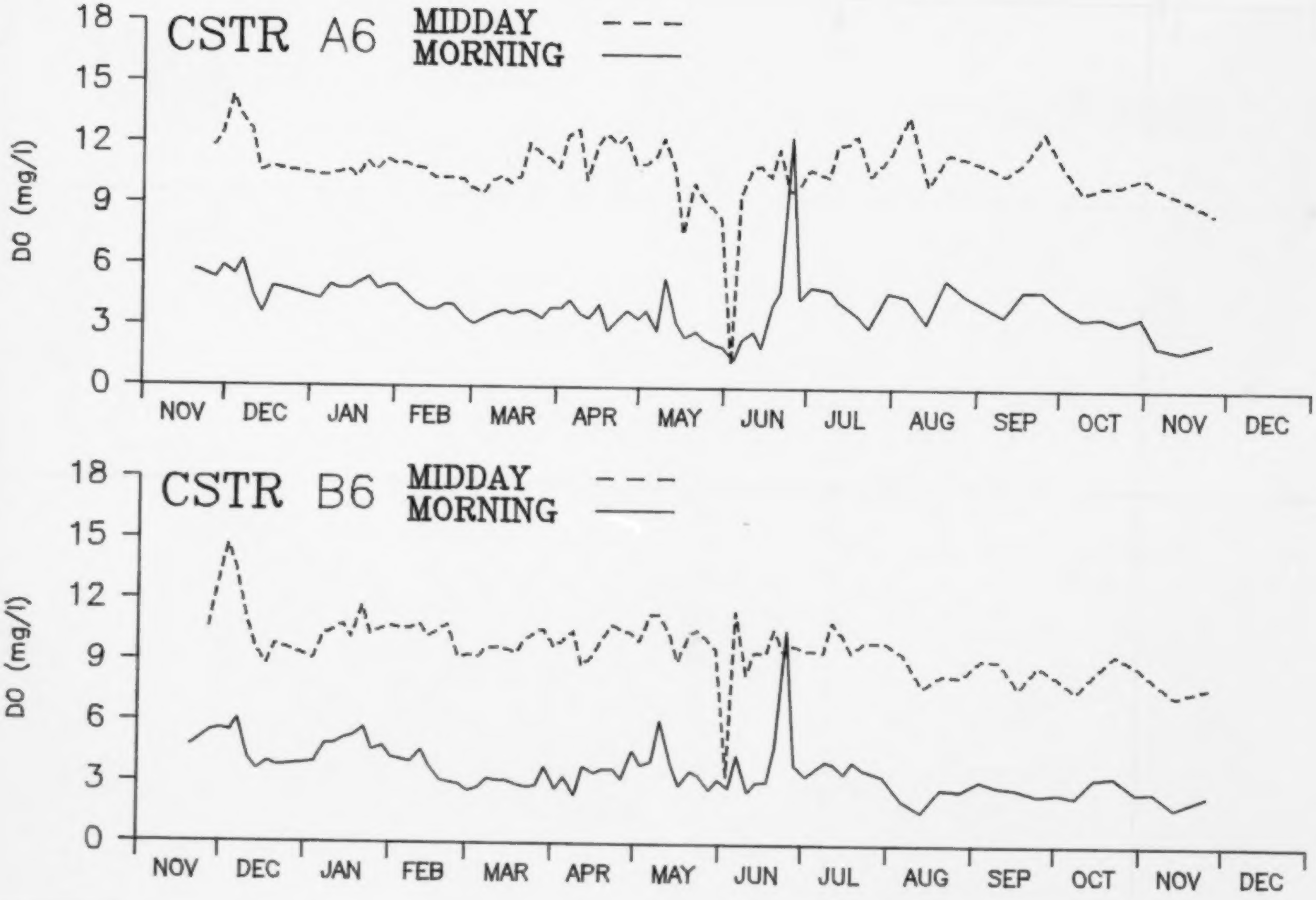


Figure 51. Dissolved Oxygen Concentrations in A6 and B6

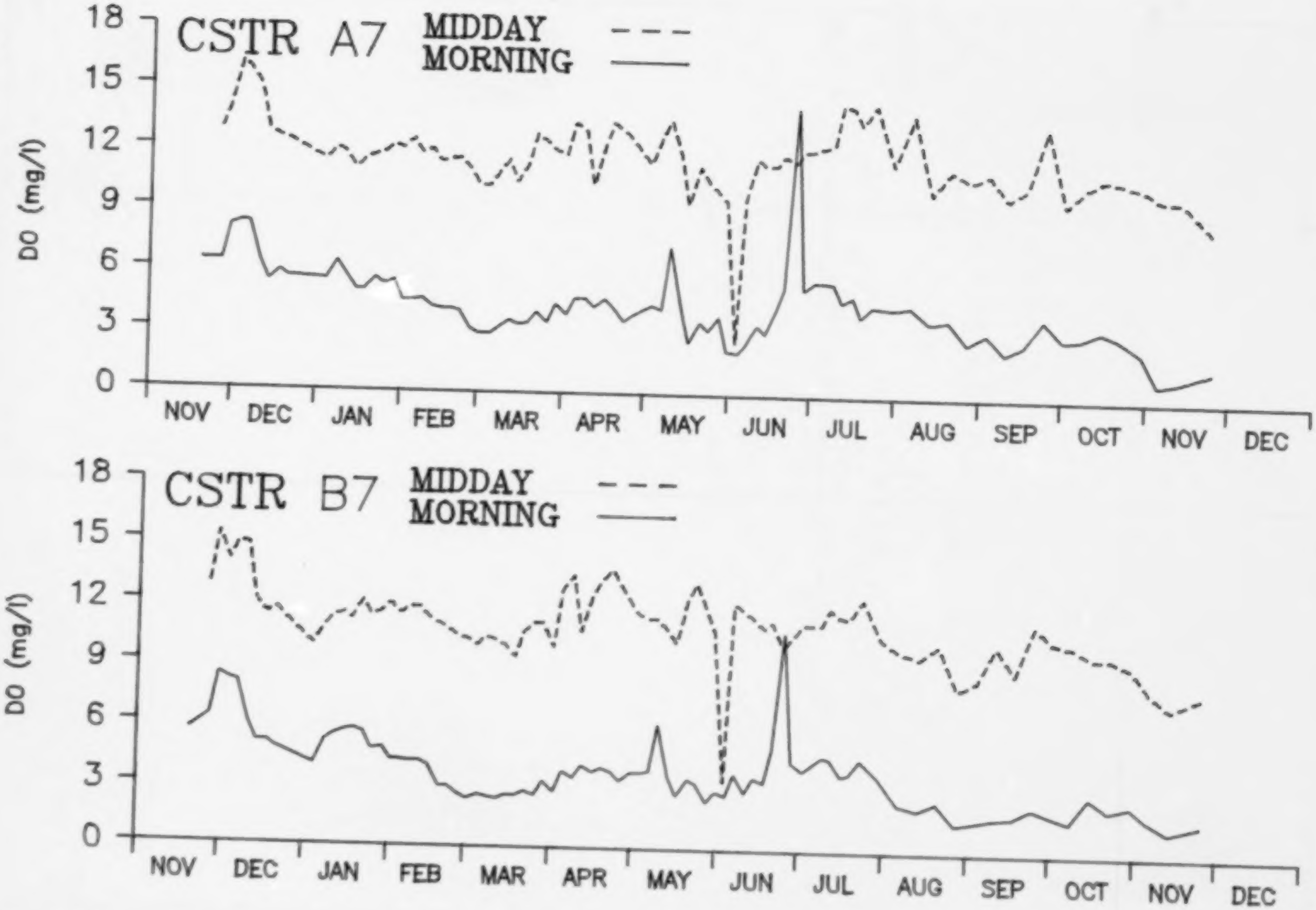


Figure 52. Dissolved Oxygen Concentrations in A7 and B7

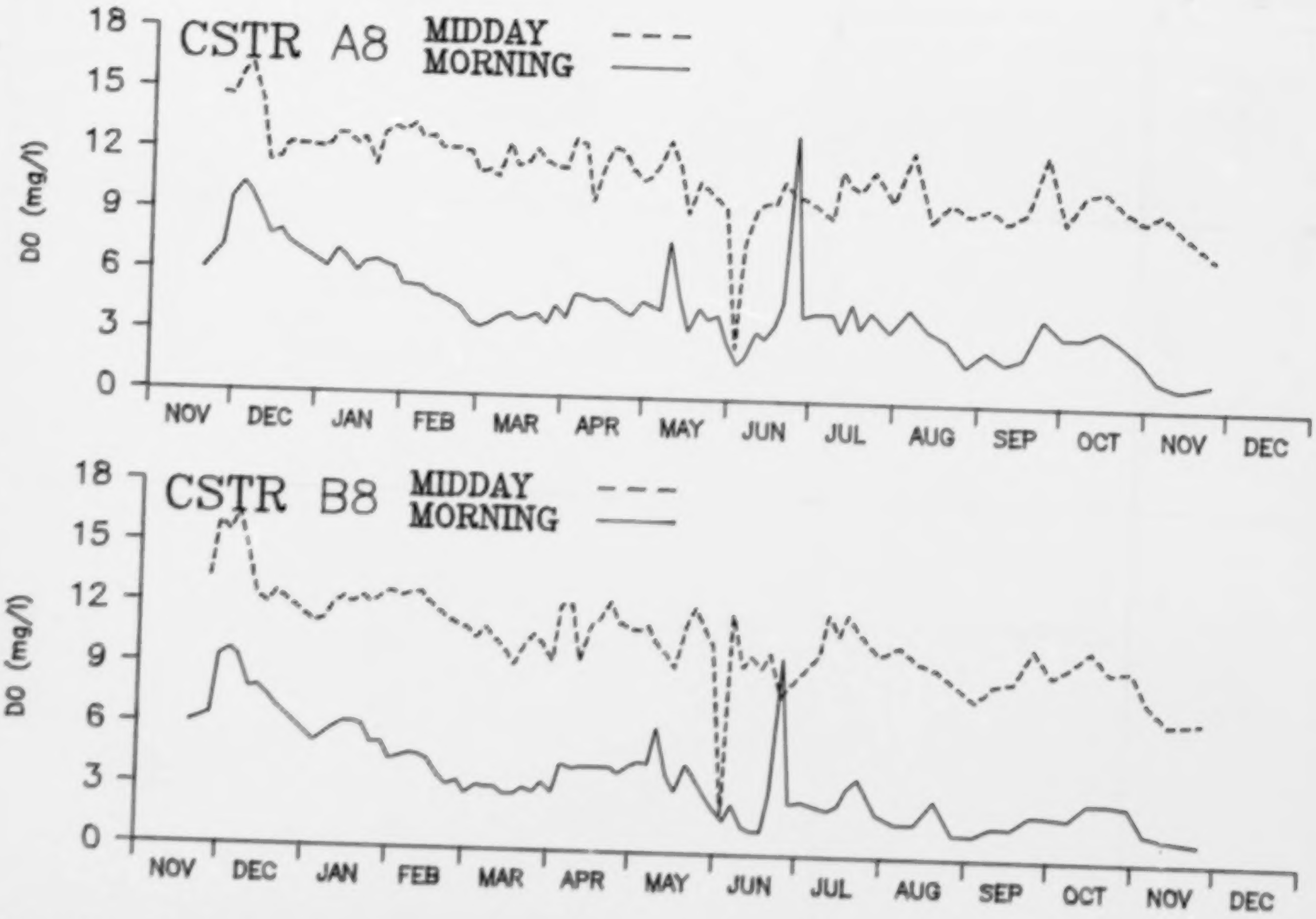


Figure 53. Dissolved Oxygen Concentrations in A8 and B8

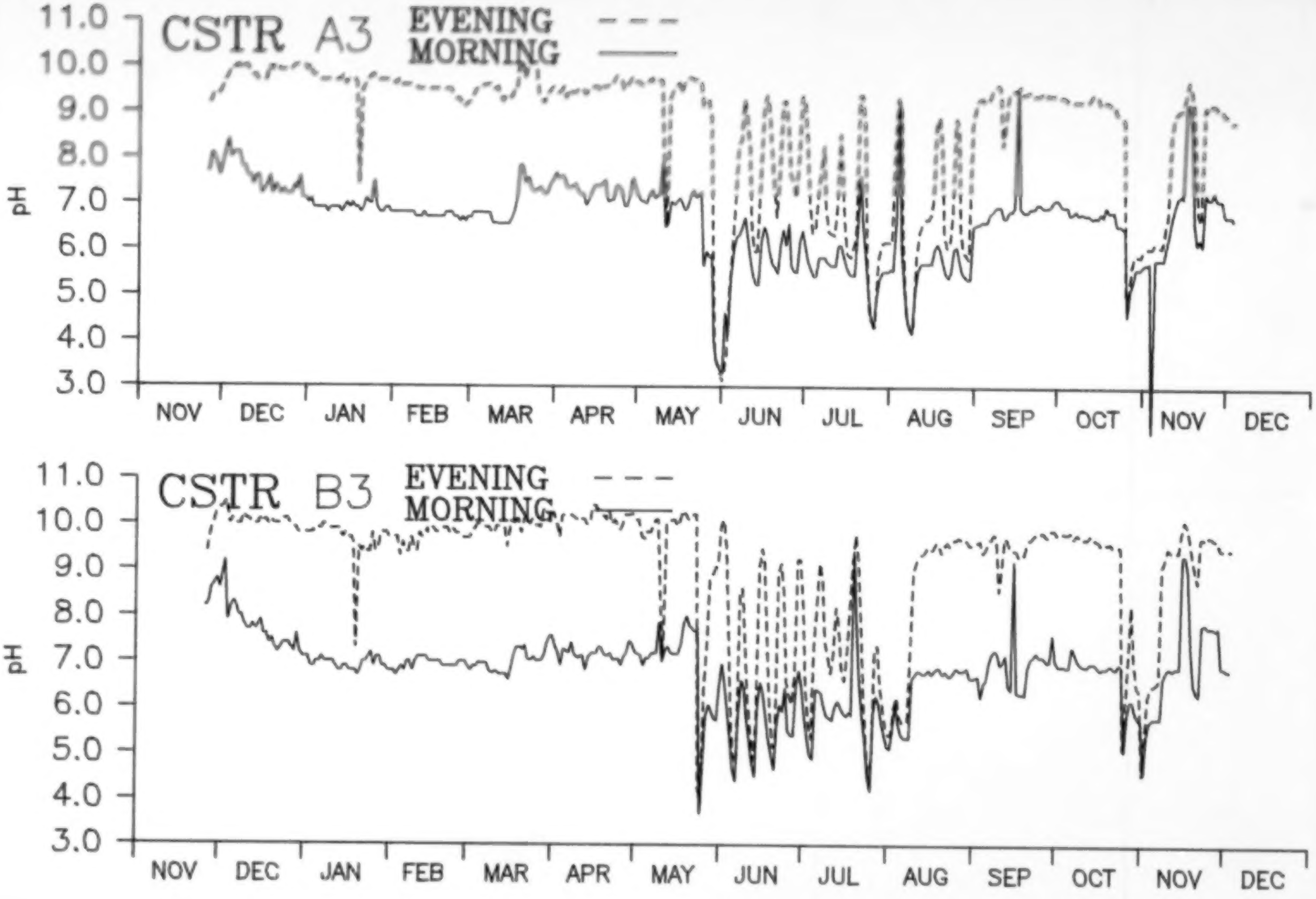


Figure 54. pH Values in CSTRs A3 and B3

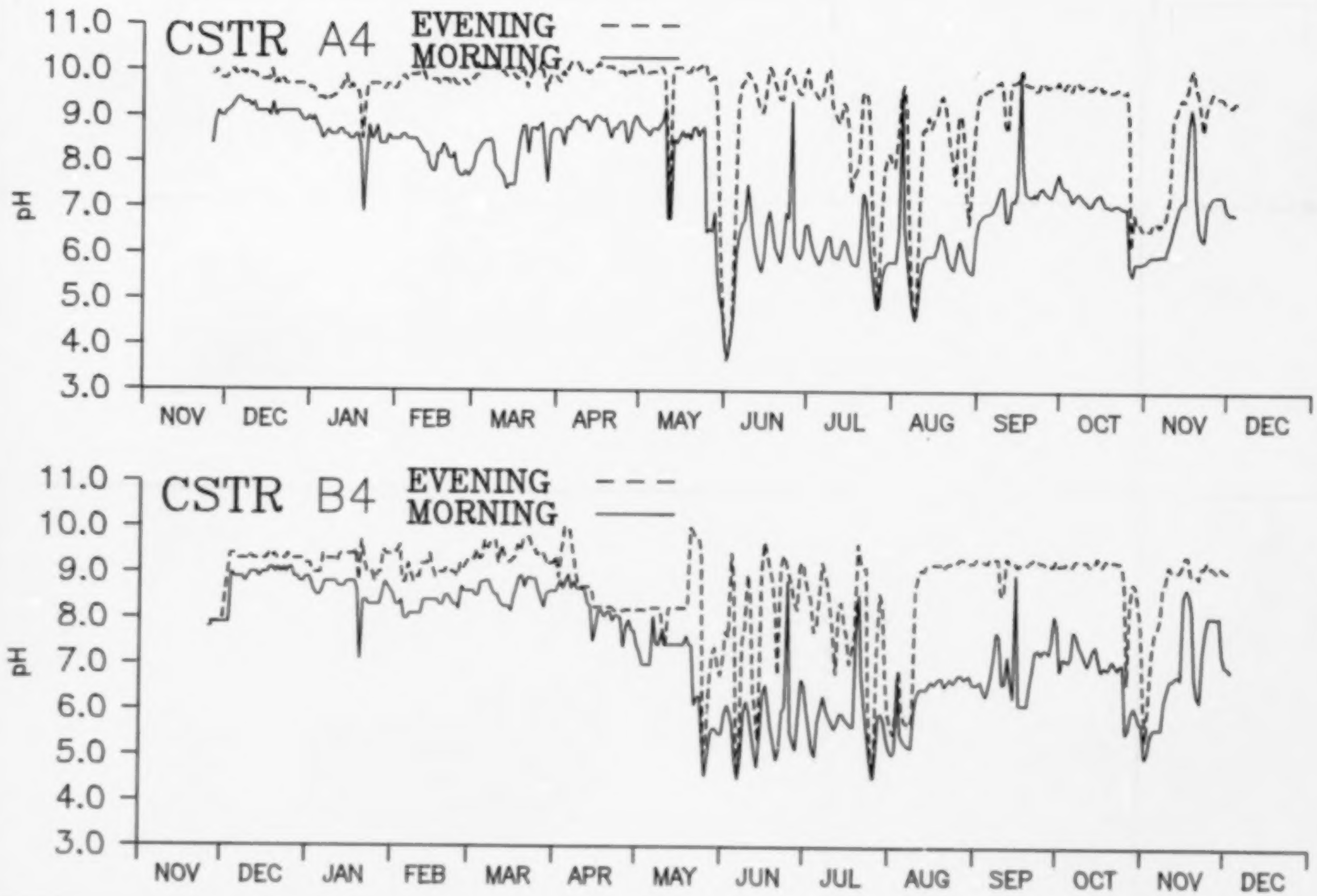


Figure 55. pH Values in CSTRs A4 and B4

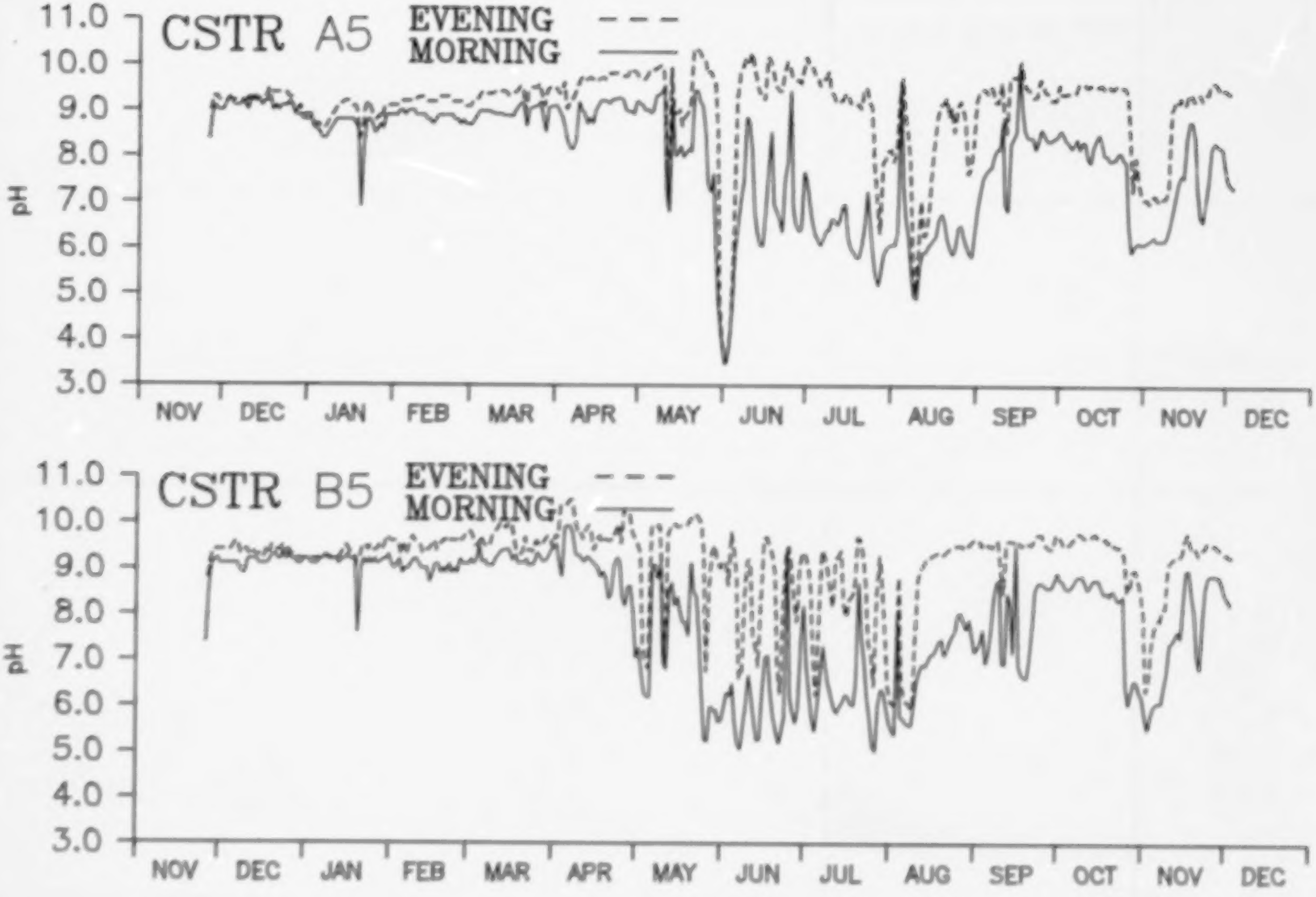


Figure 56. pH Values in CSTRs A5 and B5

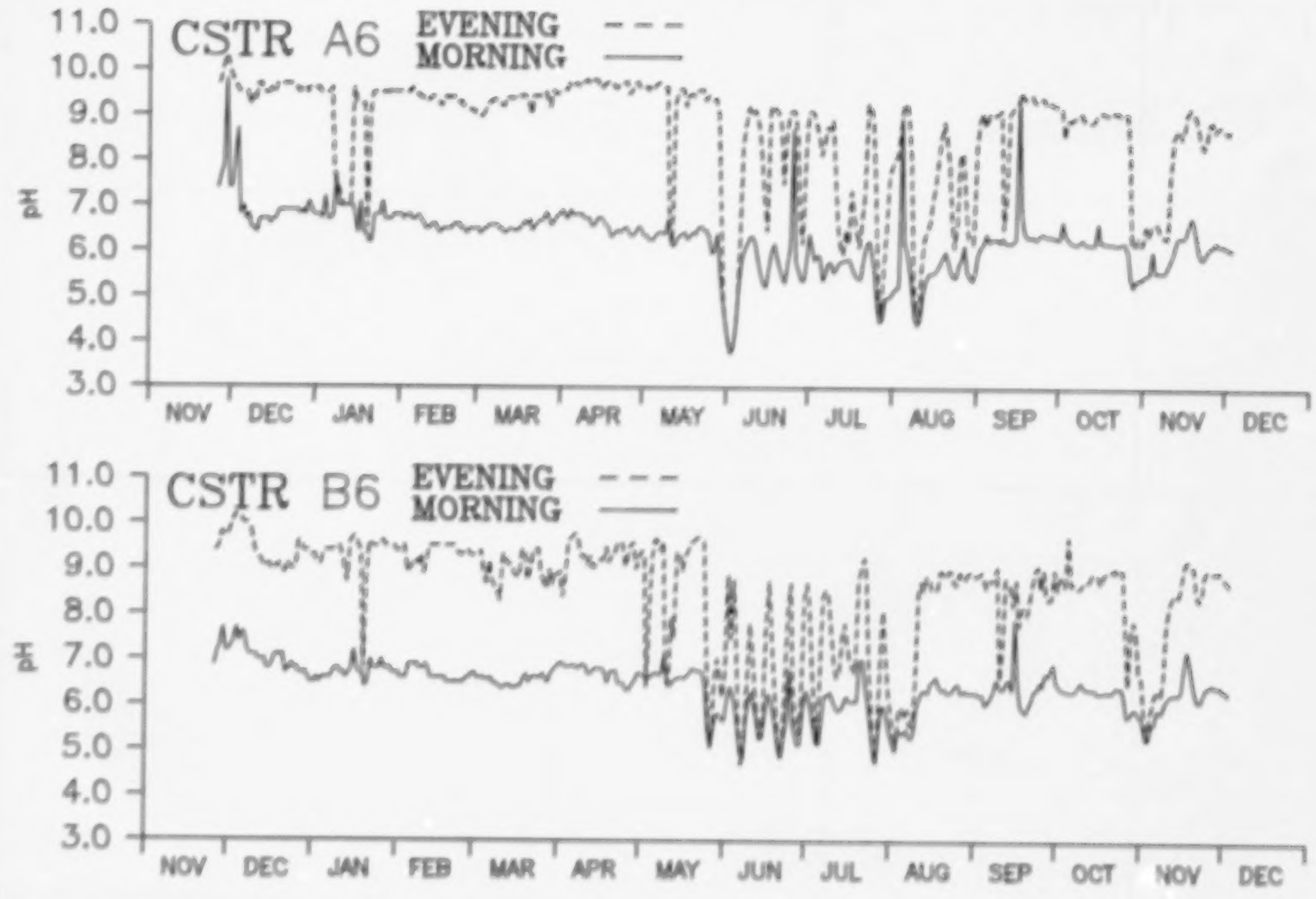


Figure 57. pH Values in CSTRs A6 and B6

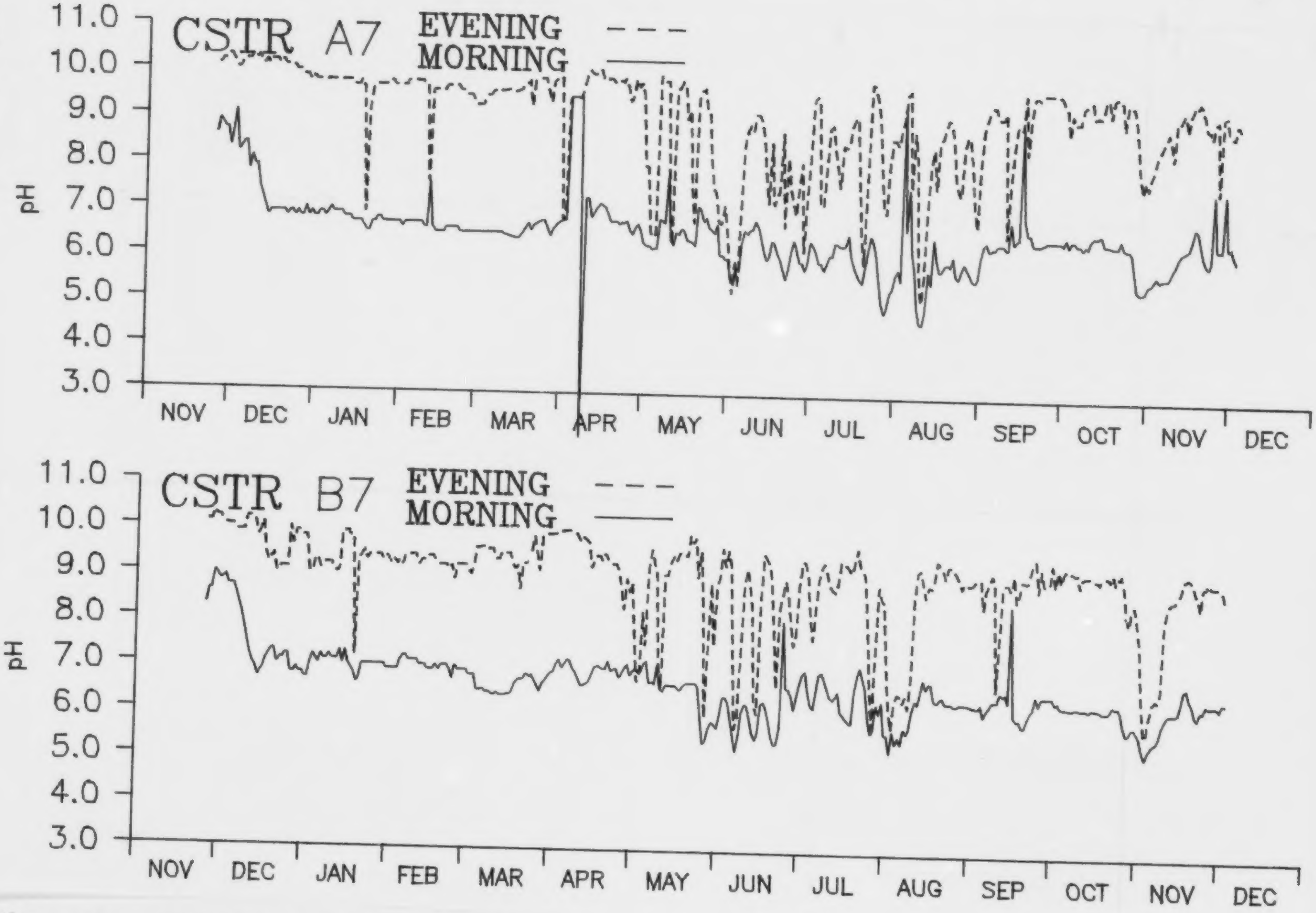


Figure 58. pH Values in CSTRs A7 and B7

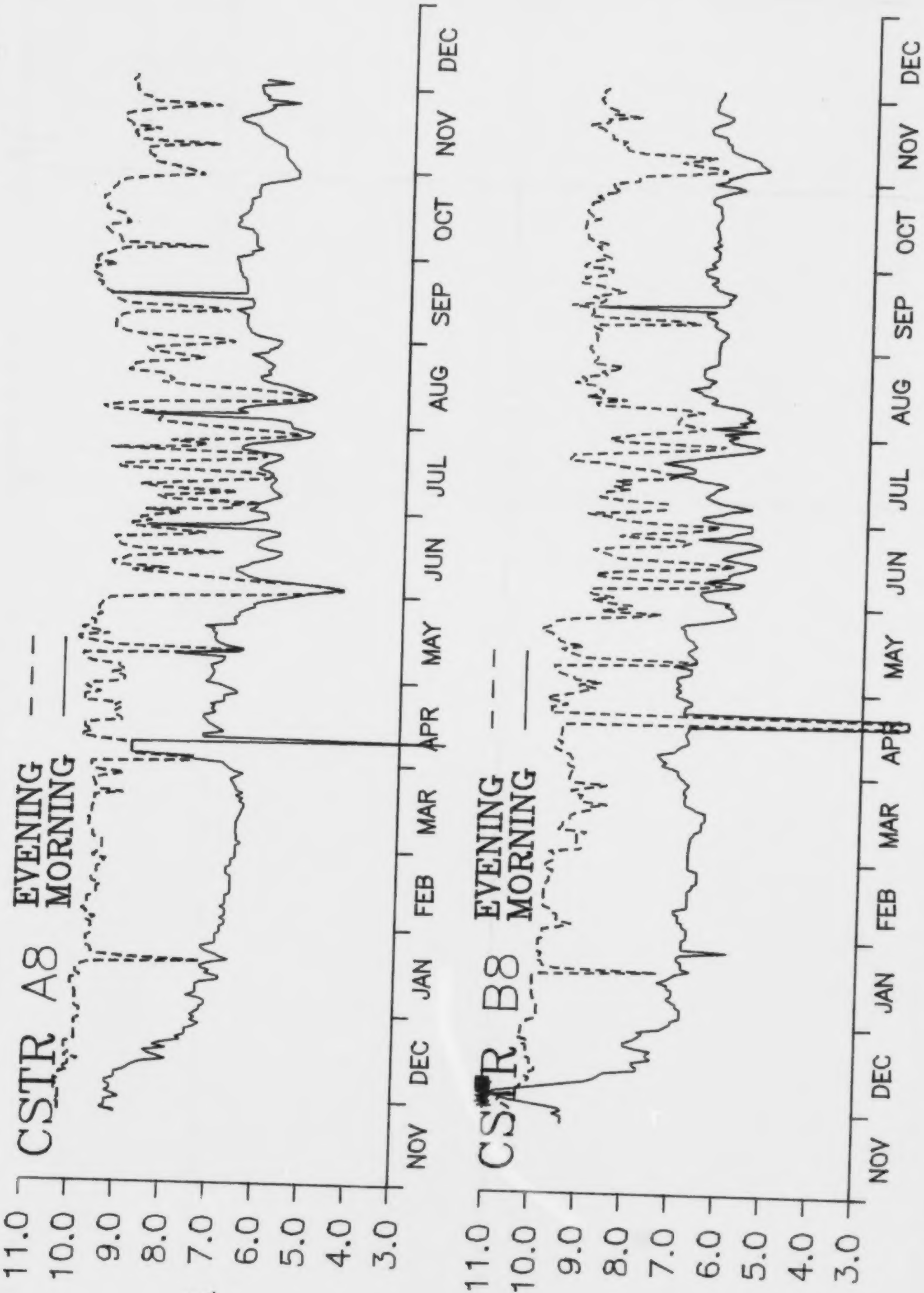


Figure 59. pH Values in CSTRs A8 and B8

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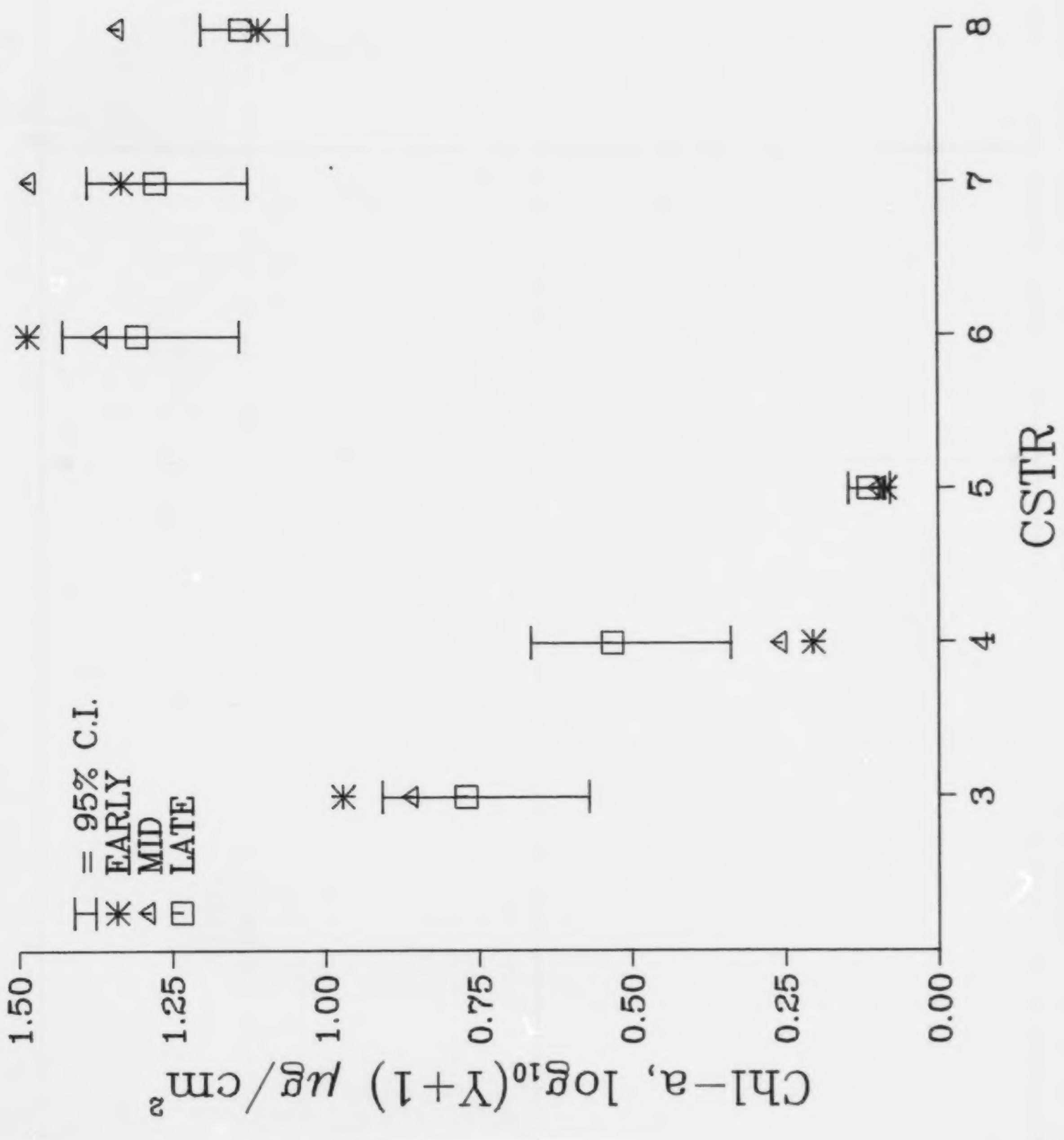

Figure 60. Aufwuchs chlorophyll a in AEcoS at three sampling times corresponding to 2 months (early), 6 months (mid), and 9 months (late) of system development

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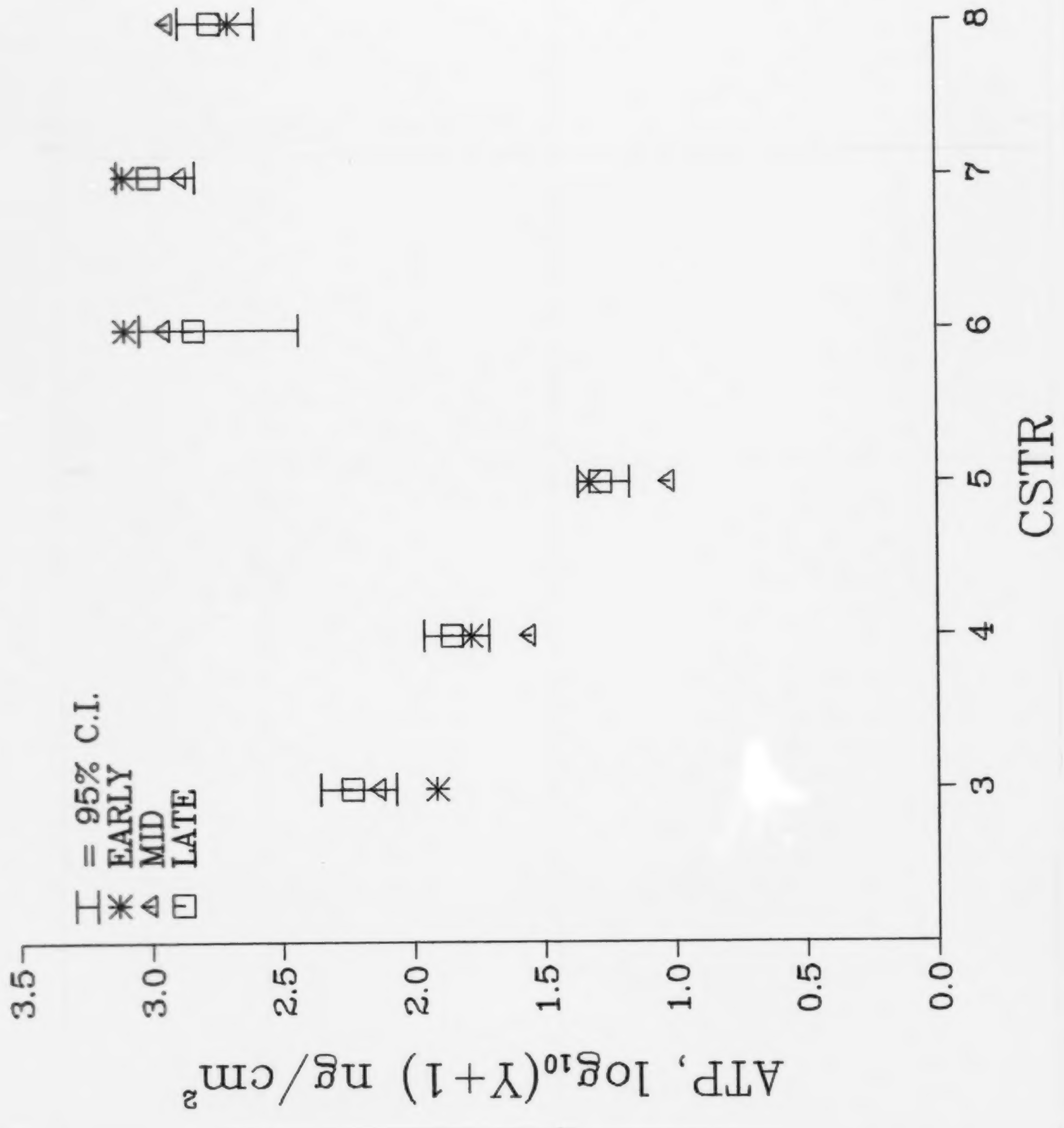

Figure 61. Aufwuchs adenosine triphosphate in AEcoS at three sampling times corresponding to 2 months (early), 6 months (mid), and 9 months (late) of system development

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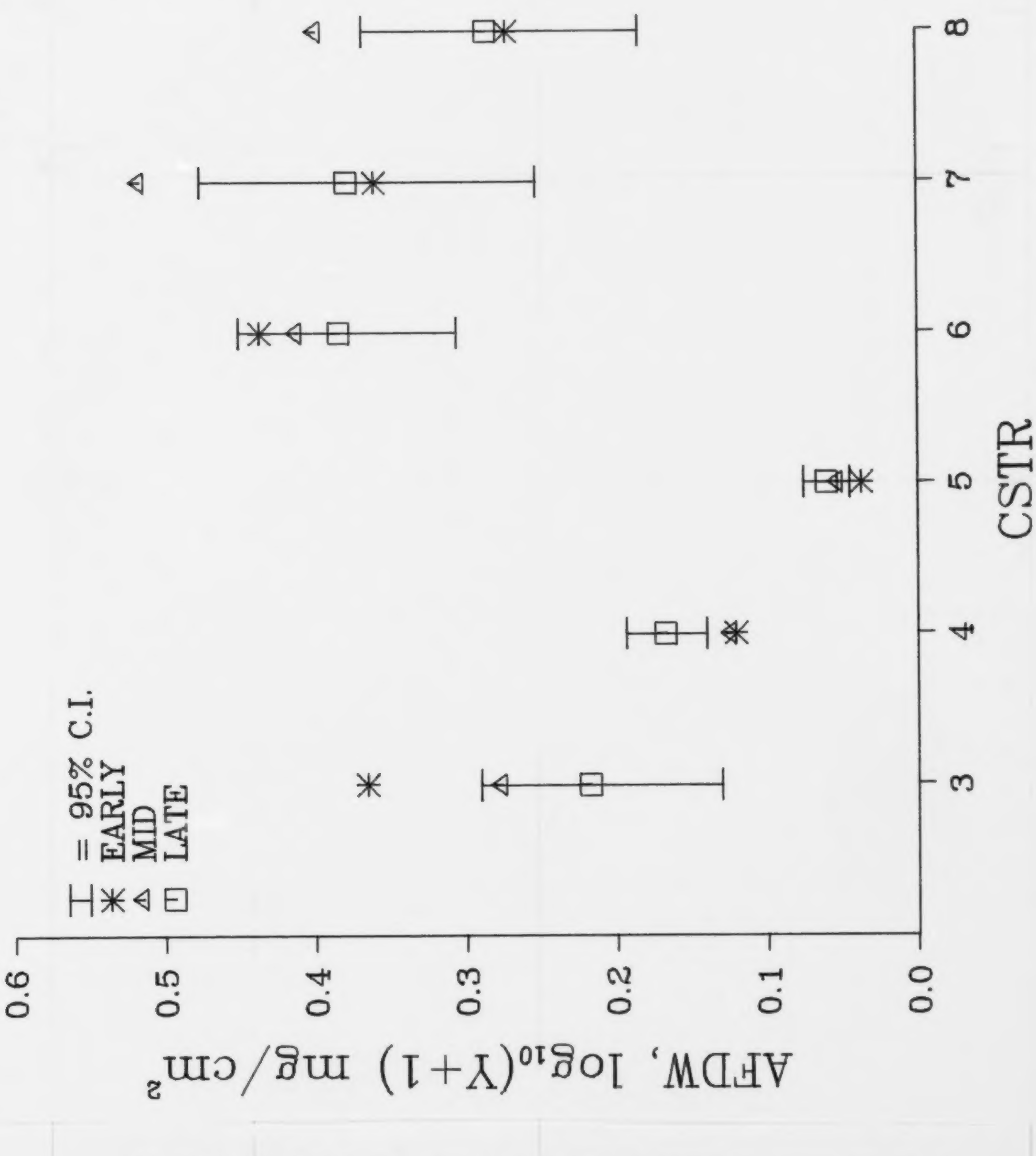

Figure 62. Aufwuchs ash-free dry weight in AEcoS at three sampling times corresponding to 2 months (early), 6 months (mid), and 9 months (late) of system development

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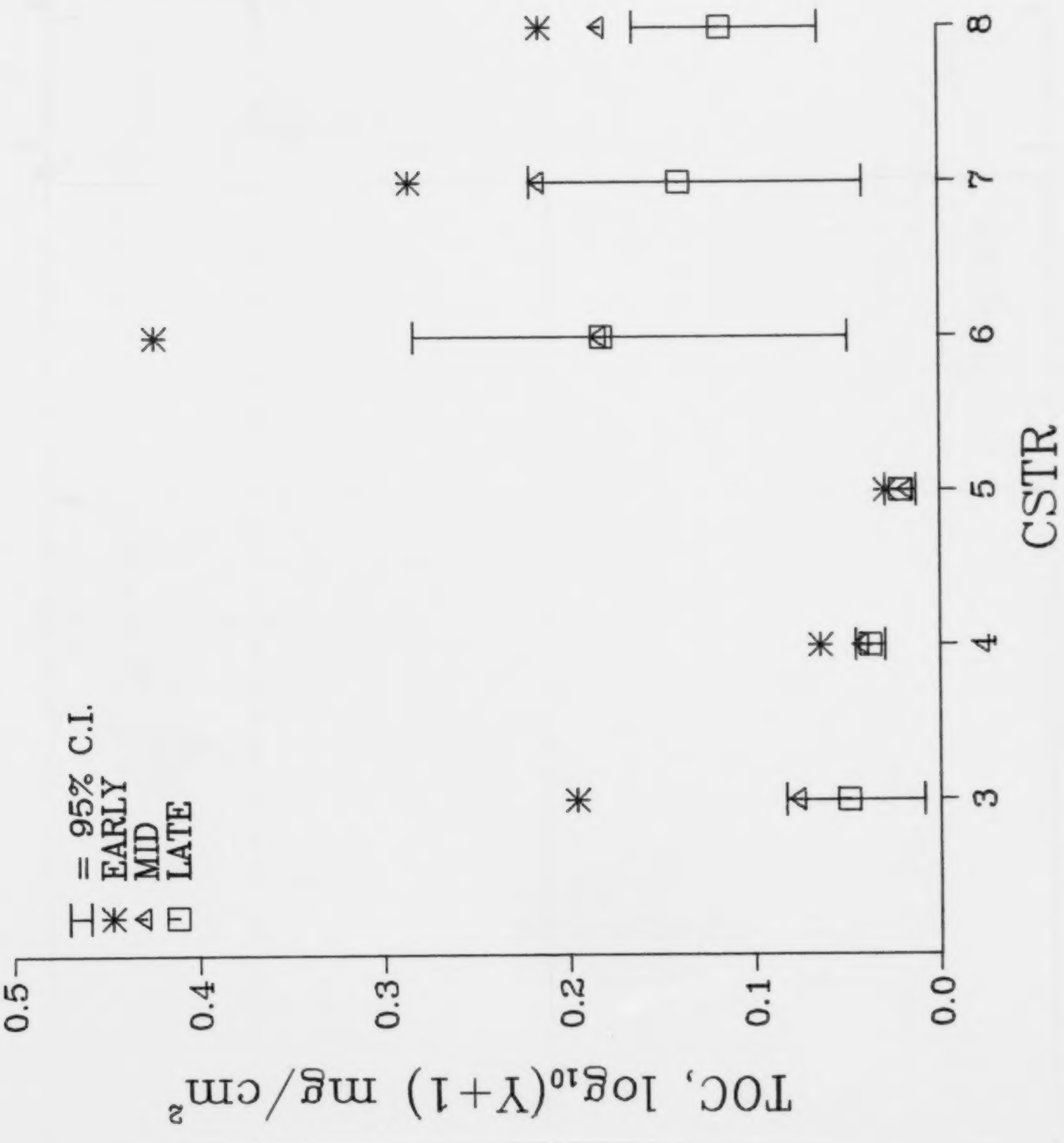

Figure 63. Aufwuchs total organic carbon in AEcoS at three sampling times corresponding to 2 months (early), 6 months (mid), and 9 months (late) of system development

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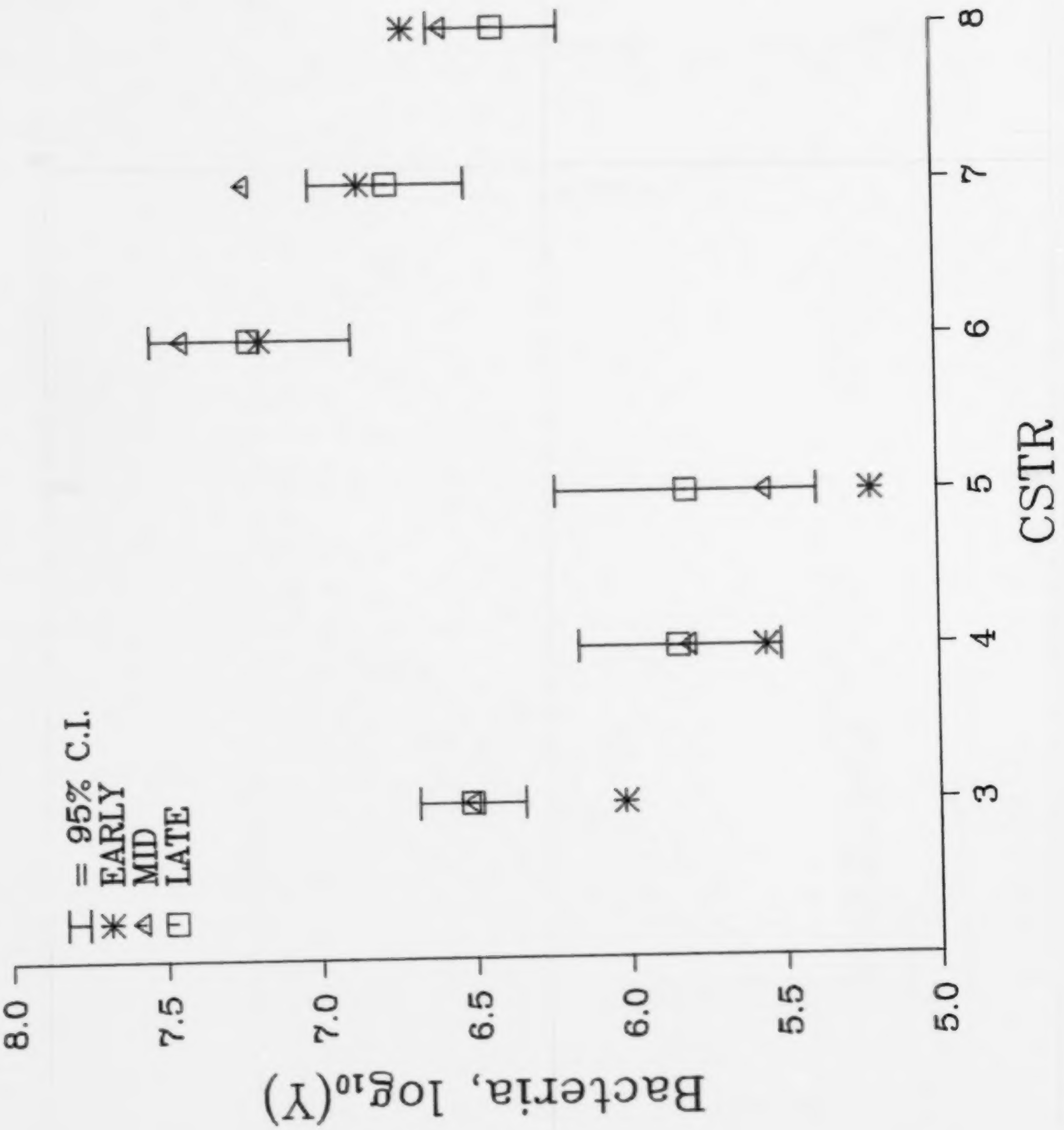

Figure 64. Aufwuchs bacterial numbers in AEcoS at three sampling times corresponding to 2 months (early), 6 months (mid), and 9 months (late) of system development

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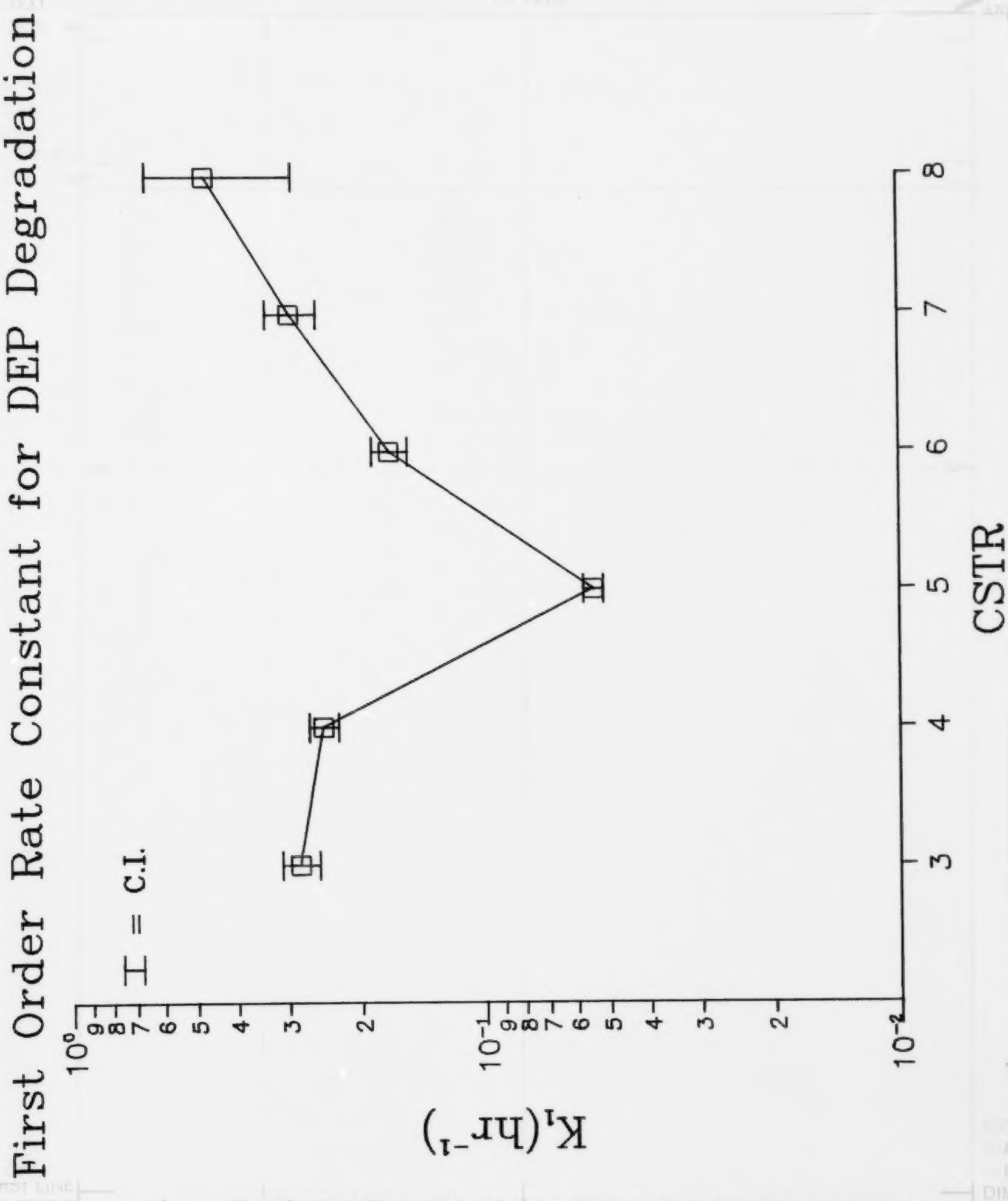

Figure 65. First order rates of degradation (k_1) for DEP in AEcoS (Channels A and B averaged) with 95% confidence intervals

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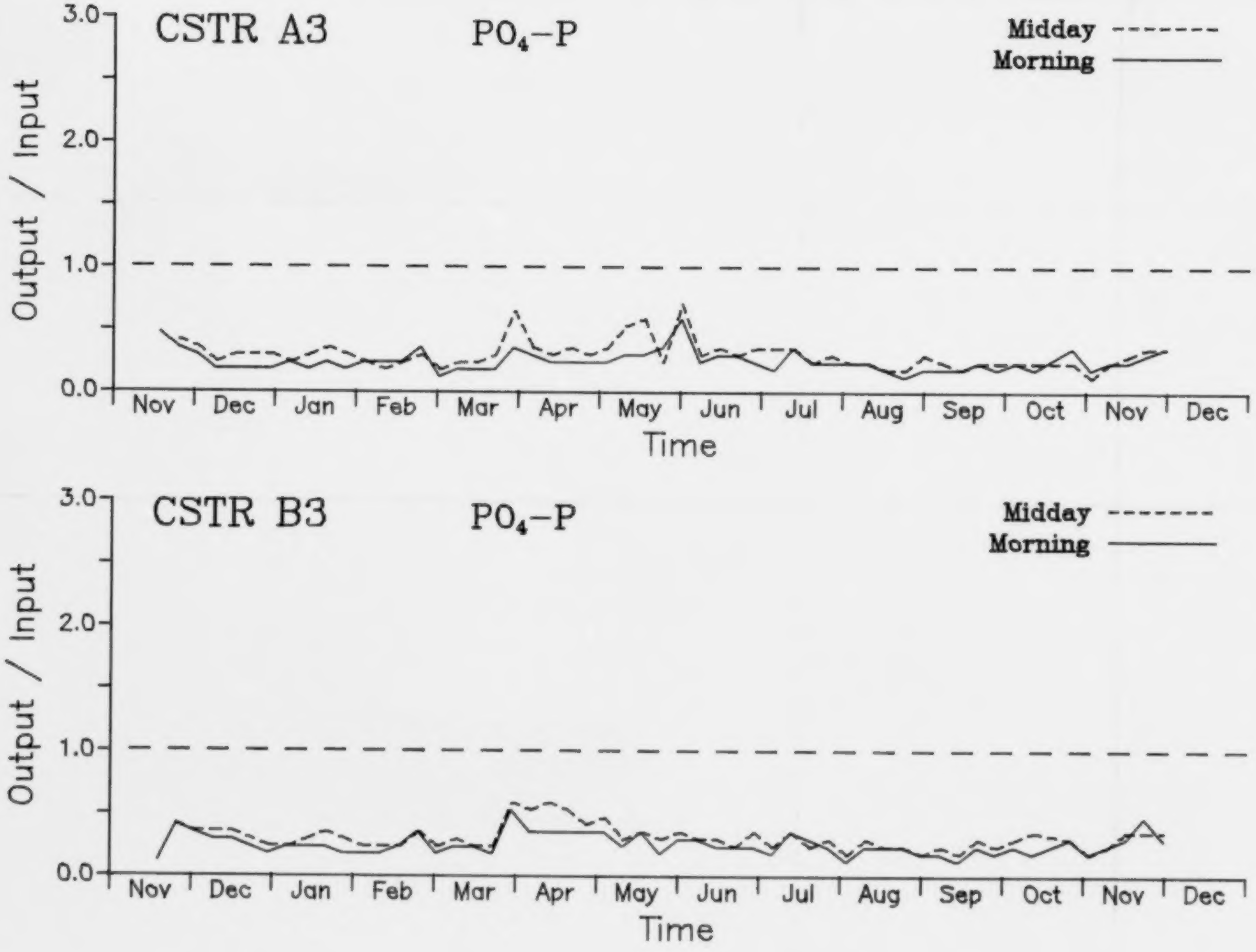

Figure 66. Orthophosphate Output:Input Ratios in CSTRs A3 and B3

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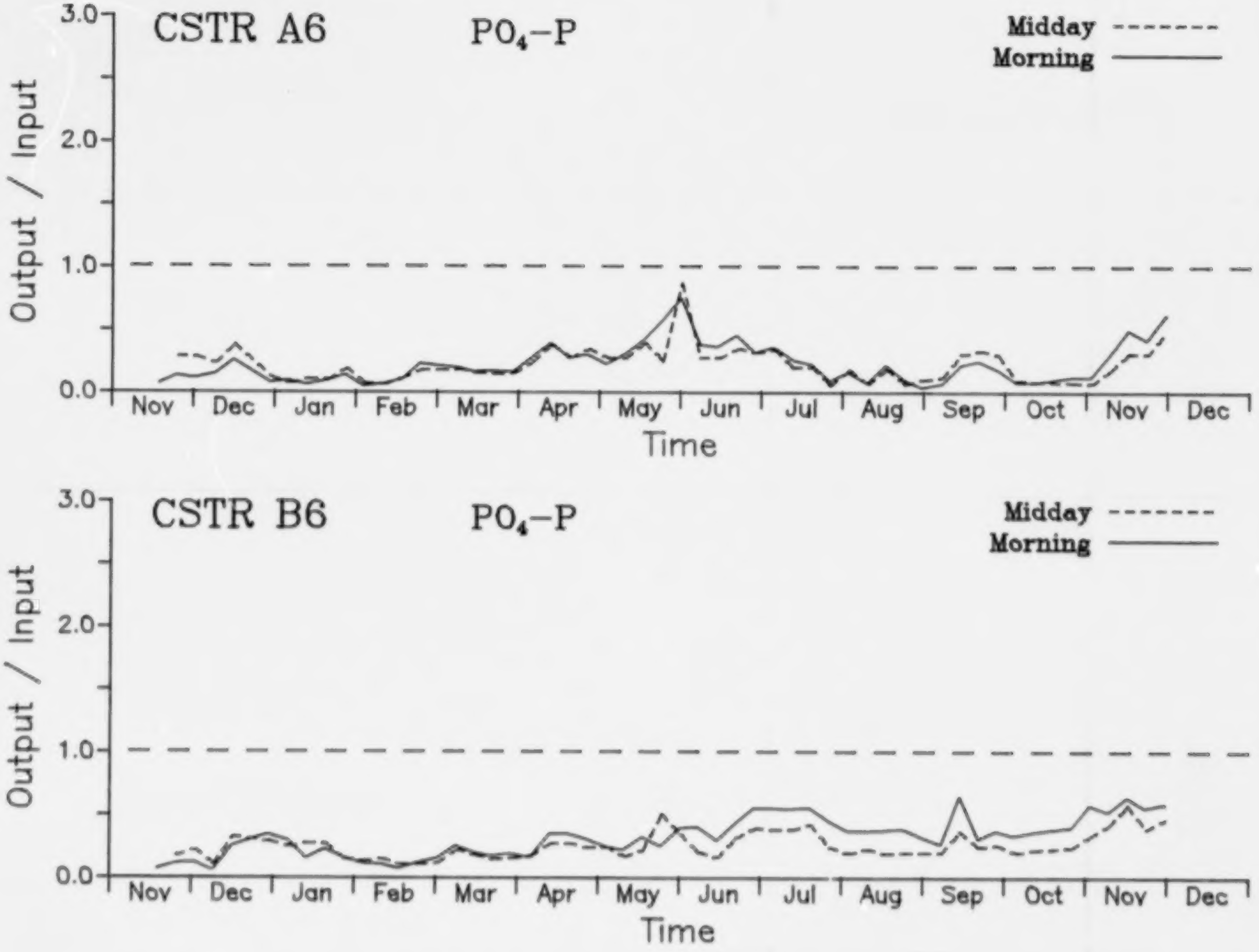

Figure 67. Orthophosphate Output:Input Ratios in CSTRs A6 and B6

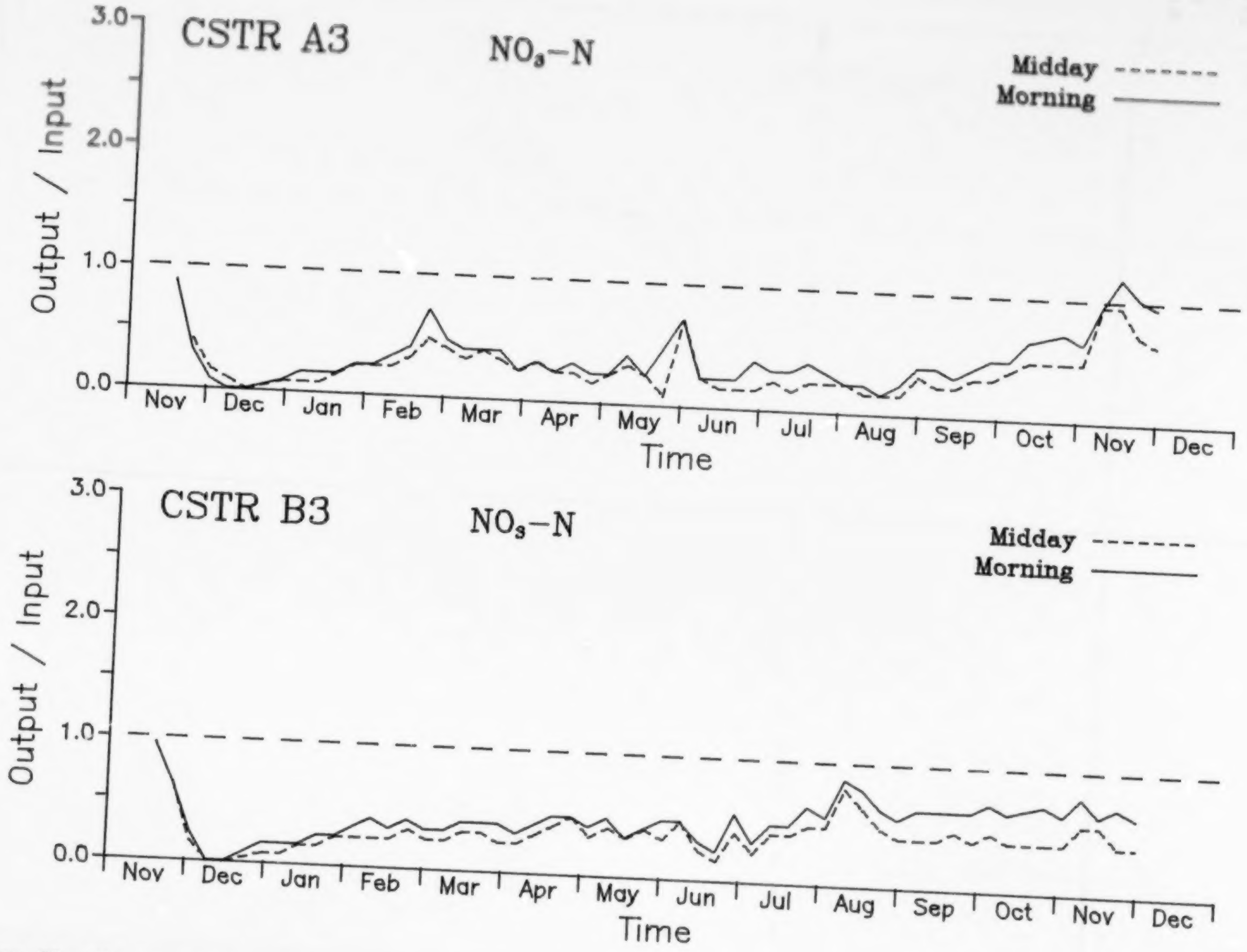

Figure 68. Nitrate-Nitrogen Output:Input Ratios in CSTRs A3 and B3

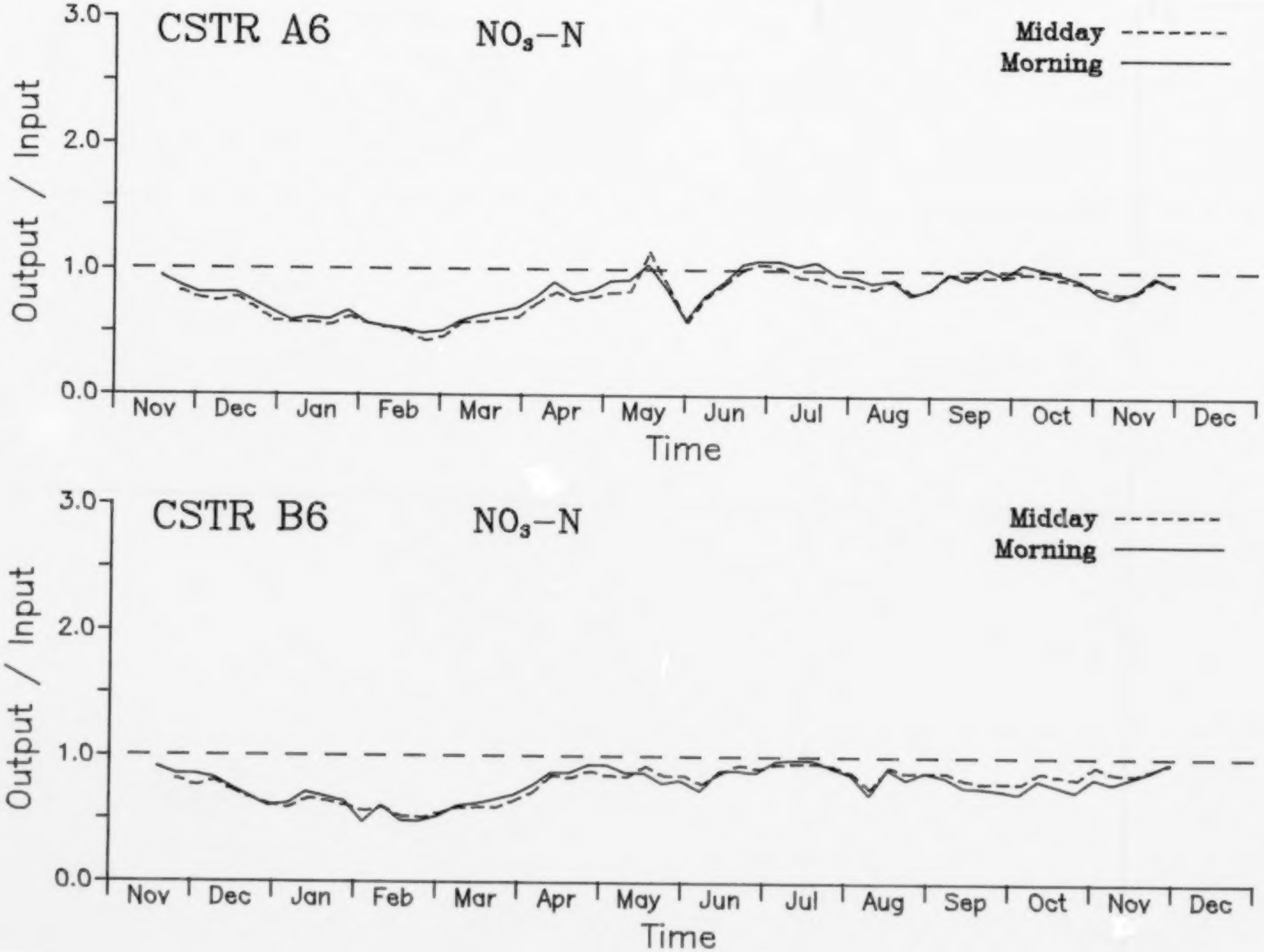

Figure 69. Nitrate-Nitrogen Output:Input Ratios in CSTRs A6 and B6

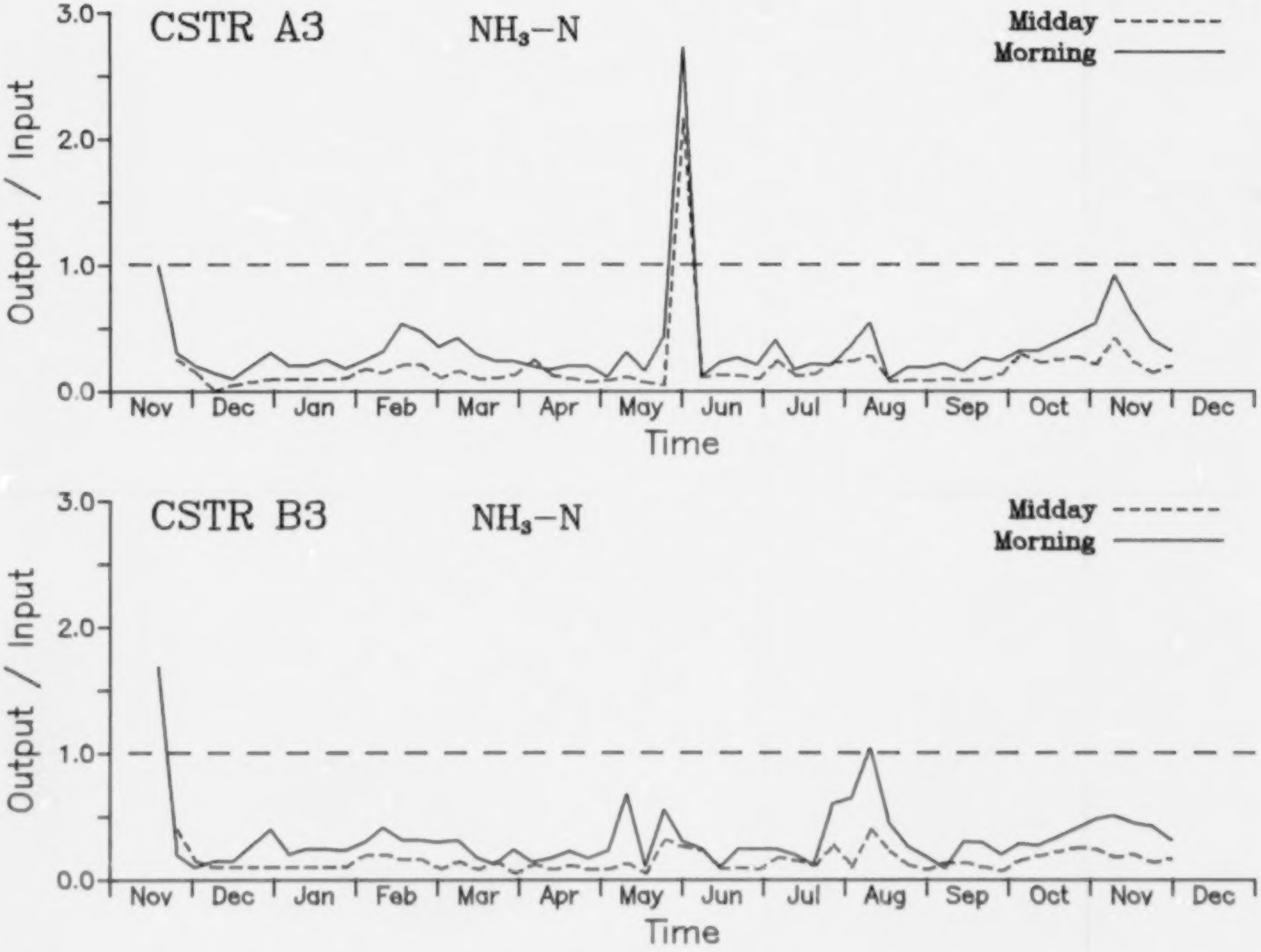

Figure 70. Ammonia-Nitrogen Output:Input Ratios in CSTRs A3 and B3

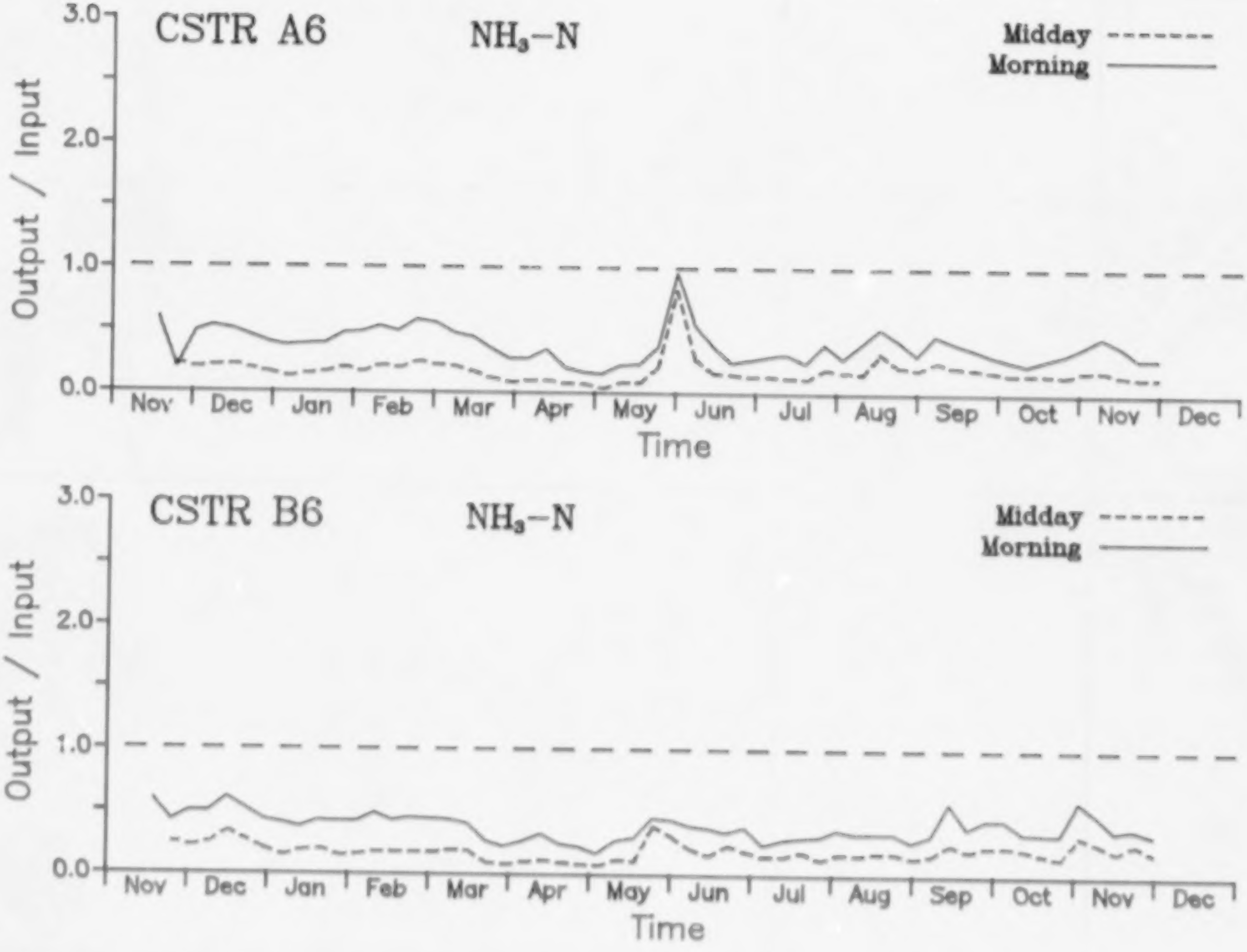

Figure 71. Ammonia-Nitrogen Output:Input Ratios in CSTRs A6 and B6

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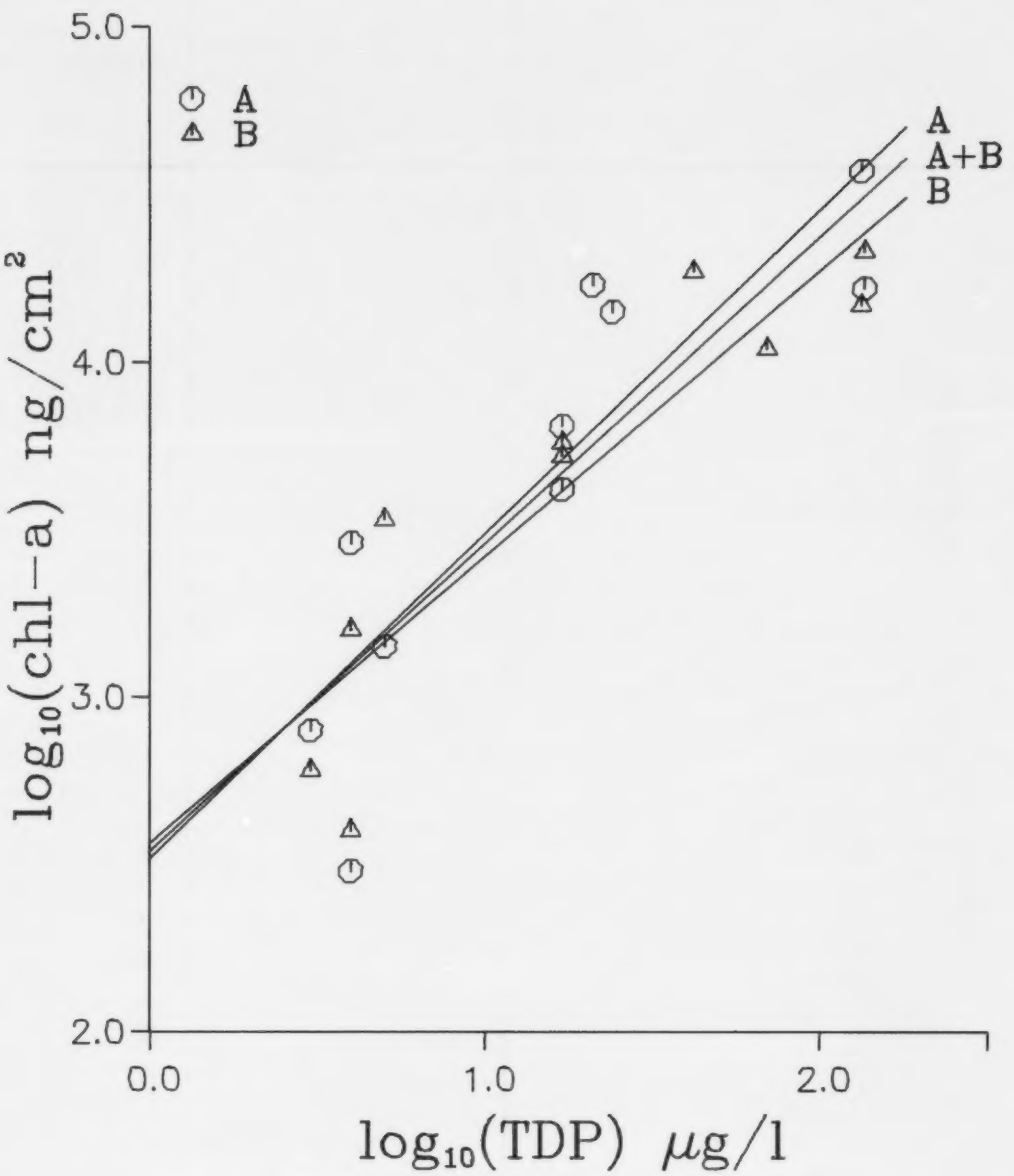

Figure 72. Linear relationship between aufwuchs chlorophyll a concentration and total dissolved phosphorus input concentration for Channel A, Channel B, and Channels A and B combined

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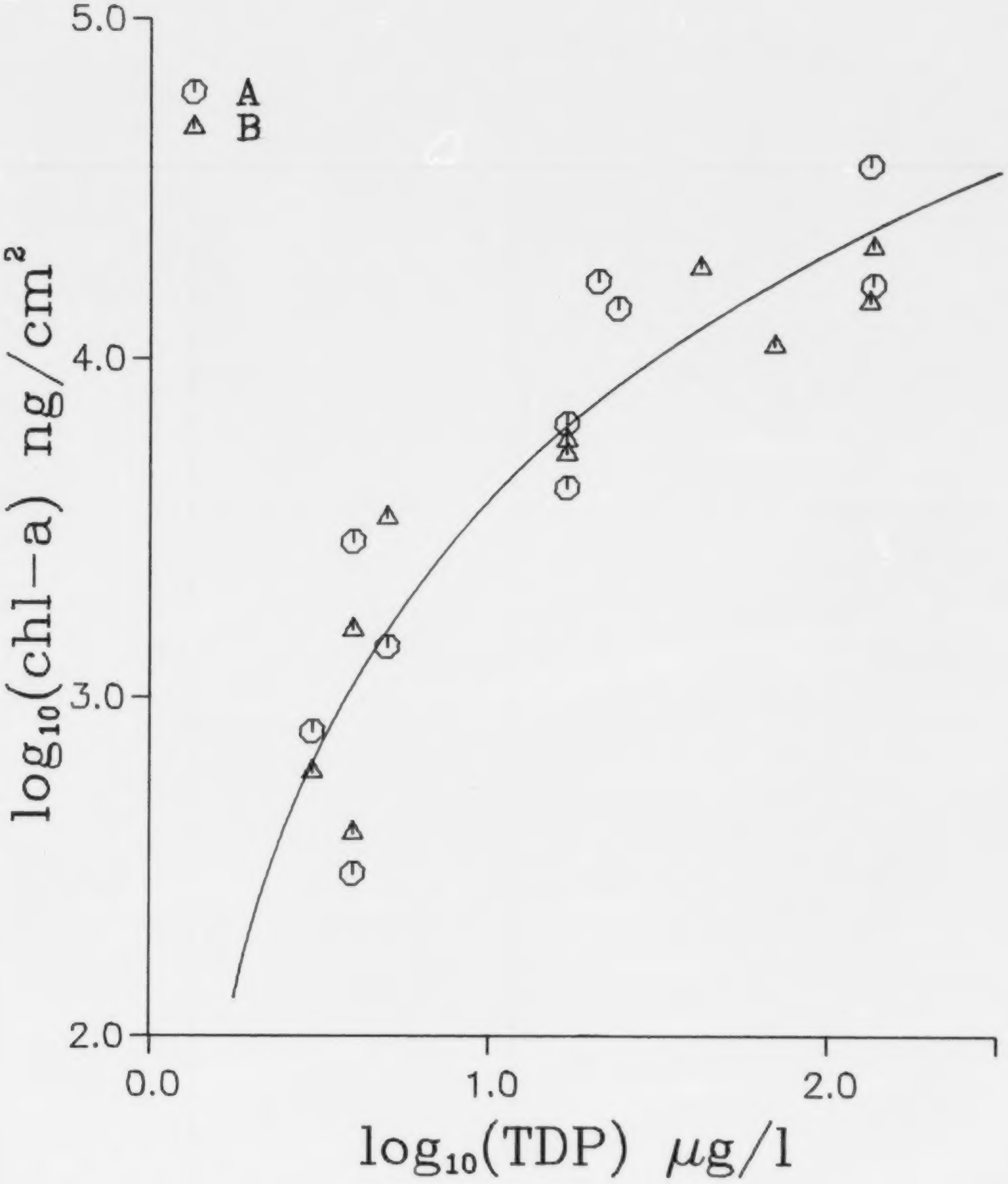

Figure 73. Curvilinear relationship between aufwuchs chlorophyll a concentration and total dissolved phosphorus input concentration for Channels A and B combined

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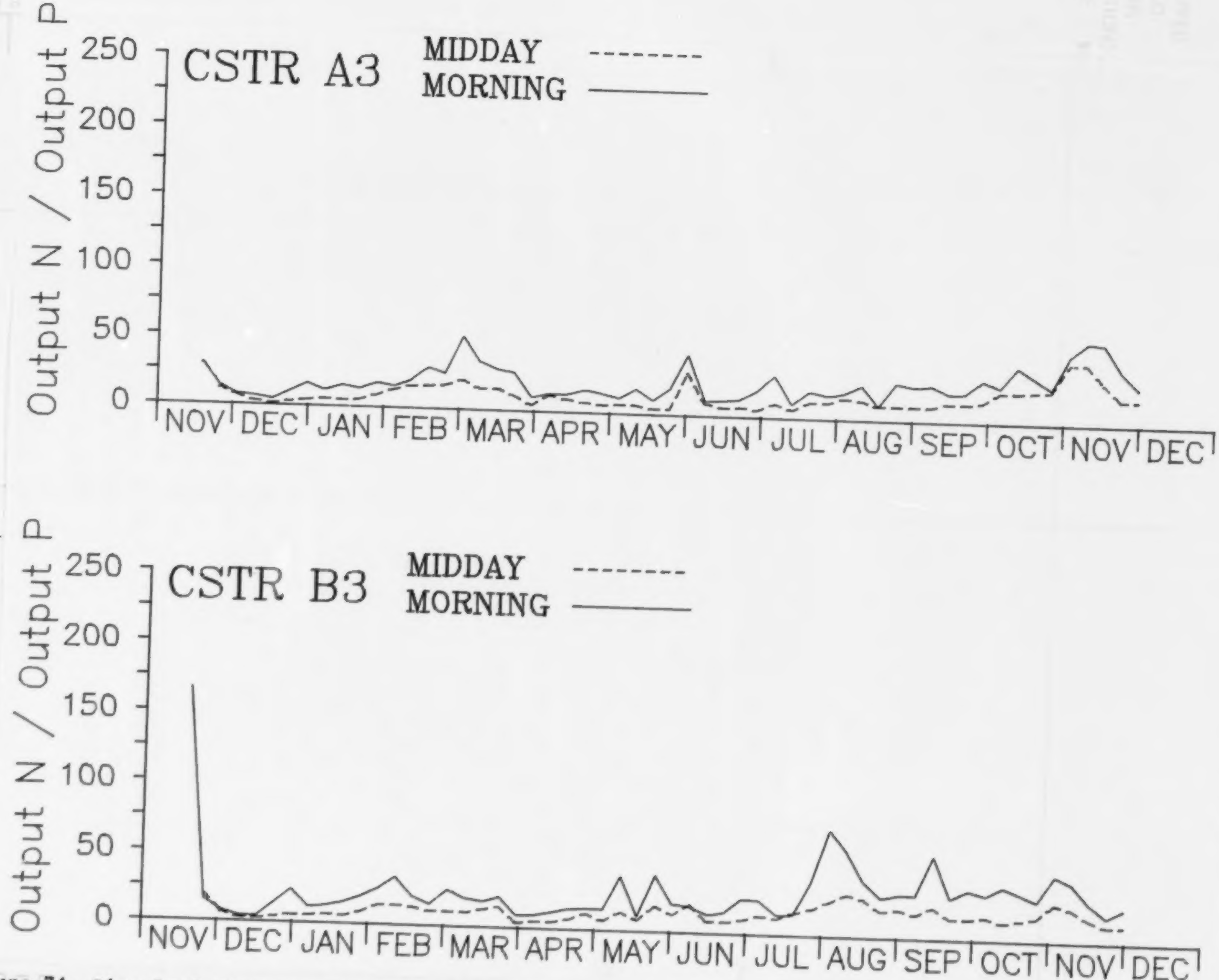

Figure 74. Dissolved Nitrogen:Phosphorus Output Ratios in CSTRs A3 and B3

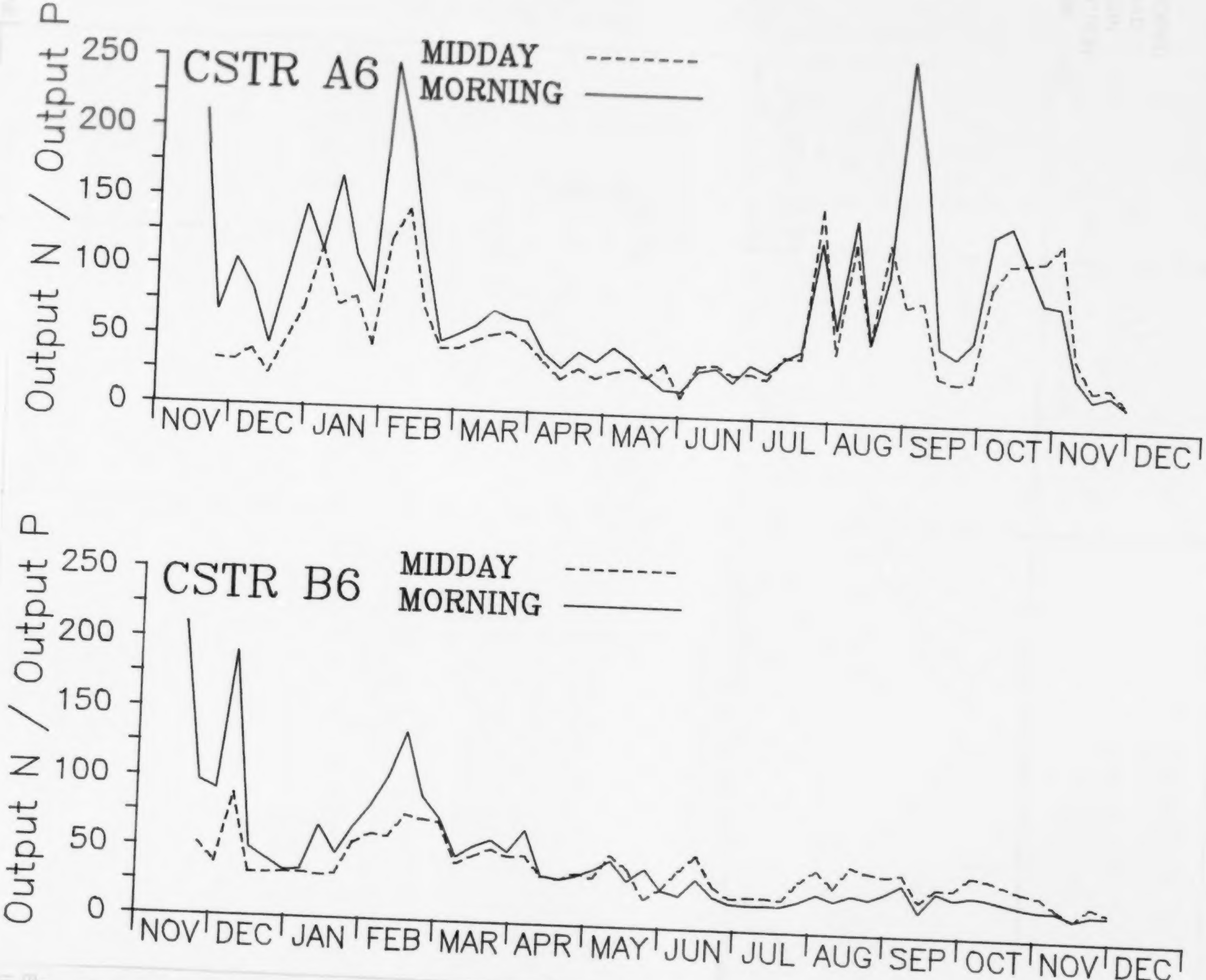

Figure 75. Dissolved Nitrogen:Phosphorus Output Ratios in CSTRs A6 and B6

Table 1. Elemental concentrations in AEcoS.

Macronutrients	mg/l	Micronutrients	ug/l
P	0.15	B	31.0
Si	2.94	Mn	11.1
HCO_3	34.50	Zn	14.9
Mg	1.33	Fe	31.4
K	2.77	Co	0.329
N	2.50	Cu	0.00354
Ca	4.82	Mo	2.75
SO_4	8.66	EDTA	440
Na	18.03[a] 26.33[b]		
Cl	17.53[c] 30.40[d] 43.27[e]		

[a] Amount added from Na salts

[b] Total Na after addition of NaOH for pH adjustment in CSTR 2

[c] Amount added prior to acid/base addition (inorganic chlorides plus HCl)

[d] Amount added through the addition of 0.610 meq/l HCl

[e] Amount added through the addition of 0.968 meq/l HCl

Table 2. Nutrient quantities in 44 l of macro-nutrient concentrate (drip rate of 4.0 ml/min into CSTR 3).

Concentrate I	Amount
$Na_2SiO_3 \cdot 9\ H_2O$	113.56 g
*$Na_2HPO_4 \cdot 7\ H_2O$	0.494 g
$NaHCO_3$	181.4 g
Concentrate II	**Amount**
$MgSO_4 \cdot 7\ H_2O$	51.52 g
K_2SO_4	23.58 g
*NH_4NO_3	2.73 g
$CaCl_2 \cdot 2\ H_2O$	67.48 g
Micronutrients	362.8 ml stock

*10% of quantity was dripped into CSTR 3, 90% into CSTR 6.

Table 3. Nutrient quantities in 1.0 l of micronutrient concentrate.

H_3BO_3	1.855 g
$MnCl_2 \cdot 4\ H_2O$	0.420 g
$ZnCL_2$	0.327 g
$Na_2EDTA \cdot 2\ H_2O$	6.0 g
$CoCl_2 \cdot 6\ H_2O$	0.014 g
$CuCl_2 \cdot 2\ H_2O$	0.001 g
$Na_2MoO_4 \cdot 2\ H_2O$	0.073 g
$FeCL_3 \cdot 6\ H_2O$	1.599 g
HCl (12 M)[a]	220 ml

[a] Amount of HCl required to neutralize Na salts of Concentrate I after addition of Concentrates I and II to channel flow

Table 4. Major sampling dates for DEP experiment.

Date	CSTRs Sampled[1]	Number Samples per CSTR	Parameter Measured[2]
11/20/79	A3-8, B3-8	1	2-4
1/8/80	A3-8, B3-8	1	1-5
1/22/80	A3-8, B3-8	1	1-5[3]
2/5/80	B3, B6	4	1-5
2/20/80	B3, B6	1	1-5
5/27/80	A3-8, B3-8	1	1-4
8/5/80	A3, B3	3	1-6
8/12/80	A4, B4	3	1-6
8/19/80	A5, B5	3	1-6
8/26/80	A6, B6	3	1-6
9/9/80	A7, B7	3	1-6
9/16/80	A8, B8	3	1-6
9/23/80	A3, B3	3	1-6
9/30/80	A4, B4	3	1-6
10/7/80	A5, B5	3	1-6
10/14/80	A6, B6	3	1-6

[1] 200 ug/l DEP introduced into each pair of CSTRs for seven days prior to sampling dates between 8/5/80 and 10/14/80

[2] Parameter code: 1 - aufwuchs TOC; 2 - suspended NH_3-N, NO_3-N, NO_2-N, DKN, PO_4-P, TDP, DOC, TOC; 3 - DO, pH; 4 - aufwuchs AFDW, Chl a, ATP, bacteria plate counts; 5 - aufwuchs algal enumeration and identification; 6 - toxicant analysis

[3] CSTRs 3 and 7 samples not taken for algal identification and enumeration

Table 5. Steady-state concentrations of DEP (ug/l) due to sorption/volatilization, photolysis, and hydrolysis of a mean input concentration of 191 ug/l DEP (s = 6.0, n = 20).

Channel	CSTR	Sorption/Volatilization			Photolysis			Hydrolysis		
		$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n
A	1	192	5.3	10	187	3.4	8	-	-	-
A	2	192	4.4	10	-	-	-	178	4.4	8
A	3	188	5.3	10	-	-	-	-	-	-
A	4	188	4.3	10	-	-	-	-	-	-
B	1	193	6.2	10	190	4.3	8	-	-	-
B	2	190	6.7	10	-	-	-	182	3.9	8
B	3	185	5.4	10	-	-	-	-	-	-
B	4	189	5.6	10	-	-	-	-	-	-

Note: Study conducted in the absence of biological growth

Table 6. Concentrations (mg/l), % degradation, and first-order degradation rate coefficients (k_1) of DEP in AEcoS.

		Concentration[1]		% Degraded[1]		$k_1 (hr^{-1})$[1,2]	
Channel	CSTR	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
A	3	0.043	0.006	77	3	0.294	0.051
A	4	0.049	0.006	73	3	0.251	0.043
A	5	0.116	0.006	37	3	0.057	0.007
A	6	0.073	0.005	60	3	0.148	0.016
A	7	0.054	0.004	71	2	0.232	0.023
A	8	0.066	0.008	64	4	0.173	0.029
B	3	0.046	0.007	75	4	0.272	0.051
B	4	0.050	0.004	73	2	0.245	0.024
B	5	0.118	0.003	36	2	0.054	0.003
B	6	0.061	0.004	67	2	0.194	0.018
B	7	0.038	0.002	79	1	0.361	0.029
B	8	0.019	0.002	90	1	0.777	0.077

[1] n = 7

[2] k_1 calculation based on a DEP mean input of 0.194 mg/l (s = 0.006, n = 22)

Table 7. Estimated chlorophyll a, ATP, and bacteria for CSTRs 4 and 8 in sediments, suspended matter, and aufwuchs during DEP additions. Numbers in parentheses indicate percentage of total found in each phase.

	Sediment[1]	Suspended	Aufwuchs[2]
Chlorophyll a (ug/l)			
CSTR 4	34.8 (8.9)	6.6 (1.7)	351 (89.4)
CSTR 8	120 (18.6)	41.0 (6.3)	484.8 (75.1)
ATP (ng/l)			
CSTR 4	2.8×10^3 (24.5)	1.4×10^2 (1.2)	8.4×10^3 (74.3)
CSTR 8	1.6×10^4 (18.7)	1.2×10^3 (1.4)	6.9×10^4 (79.8)
Bacteria (cells/l)			
CSTR 4	1.8×10^7 (12.3)	2.8×10^7 (17.4)	3.4×10^8 (81.0)

[1] Converted from a dry weight (mg) to a volume (l) basis by estimating a weight of 3000 grams for the upper 25% of the total sediment mass and dividing by CSTR volume

[2] Converted from area (cm^2) to volume (l) basis by estimating a total CSTR surface area of 3.0 m^2 and dividing by CSTR volume

Table 8. Calculated SIMI values for aufwuchs algal assemblages during DEP additions.

CSTR Comparison	Channel A			Channel B		
	All Taxa	Bluegreens	Greens	All Taxa	Bluegreens	Greens
3-4	.36	.29	.92	.85	.99	.41
3-5	.23	.27	.85	.86	.99	.23
3-6	.31	.28	.67	.67	.71	.17
3-7	.57	.80	.62	.32	.41	.17
3-8	.50	.79	.59	.09	.09	.19
4-5	.69	.99	.96	.94	.99	.81
4-6	.81	.99	.59	.59	.71	.24
4-7	.51	.48	.55	.34	.43	.23
4-8	.40	.27	.52	.09	.11	.49
5-6	.57	.99	.75	.62	.70	.32
5-7	.60	.46	.72	.31	.36	.25
5-8	.62	.24	.70	.02	.02	.47
6-7	.76	.47	.99	.39	.37	.95
6-8	.69	.25	.99	.13	.13	.72
7-8	.98	.96	.99	.69	.93	.78

Table 9. Groups of CSTRs having similar aufwuchs algal assemblages during DEP additions - Channel A[1].

All Taxa						
CSTR	3	8	7	6	5	4
Grouping		—	—	—	—	—
	—	—	—			
		—	—	—	—	
Bluegreens						
CSTR	4	5	6	7	8	3
Grouping	—	—	—	—	—	—
Greens						
CSTR	3	4	5	6	7	8
Grouping	—	—	—	—	—	—

[1] Horizontal bars group comparisons with SIMI values > 0.50

Table 10. Groups of CSTRs having similar aufwuchs algal assemblages during DEP additions - Channel B[1].

All Taxa						
CSTR	3	4	5	6	7	8
Grouping	——				——	
Blue Greens						
CSTR	3	4	5	6	7	8
Grouping	——				——	
Greens						
CSTR	3	4	5	6	7	8
Grouping	——	——		——		

[1] Horizontal bars group comparisons with SIMI values > 0.50

Table 11. Relative abundance (%) of aufwuchs algal species comprising > 1.0% of the total density on 1/22/80.

Aufwuchs Algal Species	CSTR											
	3A	3B	4A	4B	5A	5B	6A	6B	7A	7B	8A	8B
BLUE-GREENS												
Chroococcus dispersus				23.5		1.7						
Oscillatoria sp.							9.0	26.5				3.9
Lyngbya subtilus												1.8
Phormidium sp.		90.7	81.0	47.6	75.2	88.0	23.6	8.1			55.4	16.5
Phormidium subfuscum											1.2	
Aphanothece nidulans			2.3									
Aphanothece saxicola					3.4		2.8					
GREENS												
Ankistrodesmus falcatus					2.8							
Scenedesmus denticulatus							5.0					8.0
Scenedesmus dimorphus			1.5				20.3	56.1			32.2	46.8
Scenedesmus obliquus						3.4	3.1				1.2	4.4
Scenedesmus bijuga				2.7								
Stigeoclonium sp.		1.2	1.5	2.9								
Oedogonium sp.				10.4		2.6					1.2	
Coelastrum microporum		1.0	3.8	11.2	6.9		33.3					16.3
DIATOMS												
Nitzschia palea							1.6	2.9			3.7	
Synedra ulna		1.0	2.3									
Synedra incisa					2.1							
Synedra rumpens					1.4							
Achnanthes minutissima		1.3	3.8		3.4							
% of Total Density		95.2	96.2	98.3	95.2	95.7	98.7	95.1			95.0	97.7

CSTRs 3A, 7A, and 7B not sampled

Table 12. Relative abundance (%) of aufwuchs algal species comprising > 1.0% of the total density during DEP experimentation (8/5 - 9/16/80).

Aufwuchs Algal Species	CSTR											
	3A	3B	4A	4B	5A	5B	6A	6B	7A	7B	8A	8B
BLUE-GREENS												
Chroococcus dispersus	62.4	3.8		3.2				6.4	29.2	26.3	27.1	81.6
Chroococcus minor		1.6				3.0			1.2	5.1	4.8	2.7
Oscillatoria sp.							2.7	36.8				
Oscillatoria subbrevis								1.8	3.0			
Lyngbya subtilus										2.8		
Phormidium sp.	12.7	55.9	34.1	35.7	3.7	38.6	45.0	36.8	15.5	10.1	7.0	1.6
GREENS												
Chlamydomonas globosa		8.1										
Dictyosphaerium ehrenbergianum										1.3		
Ankistrodesmus falcatus	1.2	2.5	3.8	3.4	3.6	4.4		10.7		24.3		1.1
Scenedesmus denticulatus										1.5		
Scenedesmus dimorphus				1.5				1.3		3.3		
Scenedesmus bijuga	6.7		15.4		35.6	2.5	43.3		46.5	8.4	49.4	
Tetraedron minimum			1.7	3.9	2.4	12.1		1.1				
Oedogonium sp.		1.2	12.7	21.3	24.4	17.6						
Cosmarium meneghinii				2.0								
Cosmarium subreniforme						1.5						
Staurastrum sp.						4.5						
Coelastrum microporum	9.4		23.3	10.8	27.1	2.5	5.1		2.5	6.3		1.1
Zygnema sp.		13.8		10.0		2.7					5.8	
DIATOMS												
Nitzschia palea										5.6		
Navicula confervacea												5.7
Navicula minima												2.5
Gomphonema parvulum	2.0	4.4	2.6	2.2	1.4	2.0						
Cymbella minuta				1.5								
Achnanthes minutissima		3.9		2.2								
% of Total Density	94.4	95.2	93.6	97.7	98.2	91.4	96.1	94.9	97.9	95.0	94.1	96.3

Table 13. Calculated H' diversity and J evenness values for aufwuchs algal assemblages during DEP additions.

CSTR	Channel A		Channel B	
	H'	J	H'	J
3	1.97	0.47	2.72	0.70
4	2.69	0.63	2.88	0.74
5	2.27	0.58	3.14	0.68
6	1.69	0.47	2.68	0.63
7	1.97	0.57	3.13	0.74
8	2.16	0.53	1.27	0.32

Table 14. Results of Newman-Keuls Range Test for aufwuchs biomass during DEP additions (a = 0.05). Means expressed as $\log_{10}(x + 1)$, n = 6.

<table>
<tr><td colspan="7">Chlorophyll a</td></tr>
<tr><td>CSTR</td><td>6</td><td>7</td><td>8</td><td>3</td><td>4</td><td>5</td></tr>
<tr><td>Mean</td><td>1.29</td><td>1.26</td><td>1.13</td><td>0.76</td><td>0.51</td><td>0.12</td></tr>
<tr><td>Grouping</td><td colspan="2">————</td><td>——</td><td>——</td><td>——</td><td>——</td></tr>
<tr><td colspan="7">ATP</td></tr>
<tr><td>CSTR</td><td>6</td><td>7</td><td>8</td><td>3</td><td>4</td><td>5</td></tr>
<tr><td>Mean</td><td>2.98</td><td>2.77</td><td>2.74</td><td>2.21</td><td>1.84</td><td>1.27</td></tr>
<tr><td>Grouping</td><td>——</td><td colspan="2">————</td><td>——</td><td>——</td><td>——</td></tr>
<tr><td colspan="7">AFDW</td></tr>
<tr><td>CSTR</td><td>6</td><td>7</td><td>8</td><td>3</td><td>4</td><td>5</td></tr>
<tr><td>Mean</td><td>0.38</td><td>0.37</td><td>0.28</td><td>0.21</td><td>0.17</td><td>0.06</td></tr>
<tr><td>Grouping</td><td></td><td></td><td colspan="3">——————</td><td></td></tr>
<tr><td></td><td></td><td colspan="2">————</td><td></td><td colspan="2">————</td></tr>
<tr><td></td><td colspan="3">——————</td><td colspan="2">————</td><td></td></tr>
<tr><td colspan="7">TOC</td></tr>
<tr><td>CSTR</td><td>6</td><td>7</td><td>8</td><td>3</td><td>4</td><td>5</td></tr>
<tr><td>Mean</td><td>0.17</td><td>0.13</td><td>0.12</td><td>0.05</td><td>0.04</td><td>0.02</td></tr>
<tr><td>Grouping</td><td></td><td></td><td colspan="3">——————</td><td></td></tr>
<tr><td></td><td></td><td colspan="3">——————</td><td></td><td></td></tr>
<tr><td></td><td colspan="3">——————</td><td colspan="3">——————</td></tr>
<tr><td colspan="7">Bacteria</td></tr>
<tr><td>CSTR</td><td>6</td><td>7</td><td>3</td><td>8</td><td>4</td><td>5</td></tr>
<tr><td>Mean</td><td>7.21</td><td>6.76</td><td>6.51</td><td>6.41</td><td>5.84</td><td>5.81</td></tr>
<tr><td>Grouping</td><td></td><td></td><td colspan="2">————</td><td colspan="2">————</td></tr>
<tr><td></td><td>——</td><td colspan="3">——————</td><td></td><td></td></tr>
</table>

Table 15. SIMI values comparing aufwuchs algal assemblages on 1/22/80 to DEP data.

CSTR	Dates Compared	Channel A			Channel B		
		All Taxa	Bluegreens	Greens	All Taxa	Bluegreens	Greens
3[a]	2/5-8/5/80	--	--	--	.95	.99	.04
4	1/22-8/12/80	.77	.99	.78	.83	.89	.82
5	1/22-8/19/80	.12	.99	.51	.87	.99	.48
6	1/22-8/26/80	.43	.95	.10	.42	.90	.12
7[a]		--	--	--	--	--	--
8	1/22-9/16/80	.12	.24	.03	.01	.02	.25

[a] CSTRs 3 and 7 not sampled on 1/22/80

Table 16. Calculated product moment correlation coefficients for aufwuchs structural parameters during DEP additions.

	Chlorophyll a	AFDW	ATP	TOC
Chlorophyll a	1.00	0.91	0.85	0.88
AFDW		1.00	0.87	0.84
ATP			1.00	0.70
TOC				1.00

n = 60, all correlations significant ($p < .001$)

Table 17. Concentrations of trace metals* in aufwuchs from AEcoS the week of December 2, 1980.

CSTR	% Ash	ug/g dry wt			mg/g dry wt			
		Cu	Zn	Mn	Ca	Mg	Fe	Na
A3	7.99	11.5	6.3	519.3	5.6	0.8	0.3	1.3
A4	10.12	9.9	7.3	238.1	4.5	0.5	0.4	1.3
A5	9.91	8.2	6.6	220.4	5.4	0.4	0.5	2.1
A6	9.00	27.9	58.1	66.8	5.6	4.8	0.8	1.5
A7	7.59	31.0	44.7	103.4	10.9	4.0	0.6	1.8
A8	10.35	37.3	85.5	185.3	9.5	3.4	1.4	1.7
B3	7.89	14.4	18.1	293.8	8.0	1.3	0.4	2.8
B4	14.78	13.4	8.6	172.4	6.6	0.3	0.3	2.5
B5	7.92	10.3	18.0	945.3	6.9	1.0	1.3	3.3
B6	6.13	26.7	29.8	60.2	5.9	5.0	0.4	2.4
B7	6.20	36.7	43.6	64.4	8.3	3.4	0.7	1.7
B8	22.81	23.7	44.9	378.5	7.3	3.3	1.6	1.6

*Co and Mo were below detection limits

Table 18. Mean chemical concentrations (mg/l) in CSTRs over major sampling dates (see Table 4).

CSTR	DOC		PO_4-P		NO_3-N		NH_3-N	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
3	1.8	0.2	0.004	0.000	0.06	0.02	0.035	0.003
4	1.2	0.2	0.003	0.000	0.02	0.01	0.012	0.004
5	4.0	0.0	0.004	0.000	0.01	0.00	0.014	0.001
6	2.1	0.0	0.029	0.027	1.06	0.16	0.318	0.087
7	2.7	0.8	0.044	0.037	1.06	0.07	0.253	0.130
8	2.8	0.7	0.027	0.014	0.82	0.02	0.212	0.046

Table 19. Total dissolved phosphorus and aufwuchs chlorophyll a concentrations for each CSTR in AEcoS.

Period Colonized	CSTR	TDP (mg/l)		Chl a (ug/cm^2)	
		$\bar{x}$	s	$\bar{x}$	s
7/7/80-8/5/80	A3	0.017	0.002	4.2	0.7
7/7/80-8/5/80	B3	0.017	0.002	5.7	2.9
7/14/80-8/12/80	A4	0.005	0.001	1.4	0.5
7/14/80-8/12/80	B4	0.005	0.001	3.4	0.4
7/21/80-8/19/80	A5	0.004	0.001	0.3	0.1
7/21/80-8/19/80	B5	0.004	0.001	0.4	0.1
7/28/80-8/26-80	A6	0.134	0.006	16.8	5.3
7/28/80-8/26/80	B6	0.135	0.006	21.6	8.4
8/11/80-9/9/80	A7	0.021	0.008	17.1	5.3
8/11/80-9/9/80	B7	0.042	0.012	18.5	6.3
8/18/80-9/16/80	A8	0.024	0.005	14.2	0.8
8/18/80-9/16/80	B8	0.069	0.011	10.9	1.2
8/25/80-9/23/80	A3	0.017	0.002	6.4	0.3
8/25/80-9/23/80	B3	0.017	0.002	5.2	1.3
9/1/80-9/30/80	A4	0.004	0.001	2.9	0.4
9/1/80-9/30/80	B4	0.004	0.001	1.6	0.4
9/8/80-10/7/80	A5	0.003	0.001	0.8	0.1
9/8/80-10/7/80	B5	0.003	0.000	0.6	0.0
9/15/80-10/14/80	A6	0.133	0.006	37.5	18.0
9/15/80-10/14/80	B6	0.133	0.006	14.8	7.8

Table 20. Analyzed mean N:P input and output ratios (by weight) for 11/16/79 - 12/1/80.

CSTR	Sample Time	N:P Input Ratios $\bar{x}$	N:P Output Ratios				n
			Lowest	Highest	$\bar{x}$	s	
A3	morning	14.82	4.00	59.00	22.34	12.99	52
B3	morning	14.76	3.60	75.50	24.62	14.53	52
A3	mid-day	14.82	1.20	43.20	12.69	9.05	51
B3	mid-day	14.76	2.00	30.00	12.83	6.59	51
A6	morning	16.93	18.03	262.80	78.64	57.34	52
B6	morning	16.77	18.56	191.00	45.07	33.18	52
A6	mid-day	16.93	13.83	152.90	59.12	37.58	51
B6	mid-day	16.77	20.66	75.60	40.15	15.21	51

Table 21. Replicability and diel pattern Student's t-test results[1].

Nutrient	Timeblock[2]	A versus B		Morning versus Mid-day	
		CSTR 3	CSTR 6	CSTR 3	CSTR 6
NH_3-N	a	n.s.	n.s.	*	*
	b	n.s.	n.s.	*	n.s.
	c	n.s.	n.s.	n.s.	*
	d	n.s.	n.s.	*	*
NO_3-N	a	n.s.	n.s.	n.s.	n.s.
	b	n.s.	n.s.	*	n.s.
	c	n.s.	n.s.	n.s.	n.s.
	d	n.s.	*	*	n.s.
PO_4-P	a	n.s.	*	*	*
	b	n.s.	n.s.	*	n.s.
	c	*	n.s.	n.s.	n.s.
	d	n.s.	*	n.s.	*

[1] n.s. = not significant at $\alpha = 0.05$; *significant at $\alpha = 0.05$

[2] a = 11/27/80 to 2/20/80
b = 2/21/80 to 5/20/80
c = 5/21/80 to 7/20/80
d = 7/21/80 to 12/5/81

Table 22. Results of Student's t-test comparing aufwuchs biomass parameters in Channels A versus B during DEP additions.

	CSTR					
Parameter	3	4	5	6	7	8
Chlorophyll a	n.s.	*	n.s.	n.s.	n.s.	*
ATP	n.s.	*	*	n.s.	n.s.	*
AFDW	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
TOC	n.s.	n.s.	n.s.	n.s.	*	*
Bacteria	*	*	*	*	n.s.	*

* = significantly different at $\alpha = 0.05$; n.s. = not significantly different (n = 6)

Table 23. SIMI values comparing aufwuchs algal species composition in Channels A and B during DEP additions.

	SIMI Value		
CSTR	All Taxa	Bluegreens	Greens
3	.31	.33	.09
4	.86	.99	.66
5	.33	.99	.54
6	.54	.74	.11
7	.60	.97	.34
8	.48	.96	.15